VOLUME 66 *October, 1952* NUMBER 4

THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 19, N.Y.

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Percentages of Recommended Daily Intake of Eight Essential Amino Acids and of Protein Contributed by 6 Oz. of Cooked Meat*

Essential Amino Acids	Beef[3]	Lamb[4]	Pork[4]
L-Isoleucine	141	121	127
L-Leucine	150	120	125
L-Lysine	202	163	172
L-Methionine	42	34	40
L-Phenylalanine	70	63	70
L-Threonine	160	169	183
L-Tryptophan	90	90	100
L-Valine	136	107	113
Protein	56	49	51

*In the calculations, averages of the percentages of protein in six different cuts of each type of cooked meat were used, as were averages of the percentages of the amino acids in the protein of the respective cuts.

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REFERENCES

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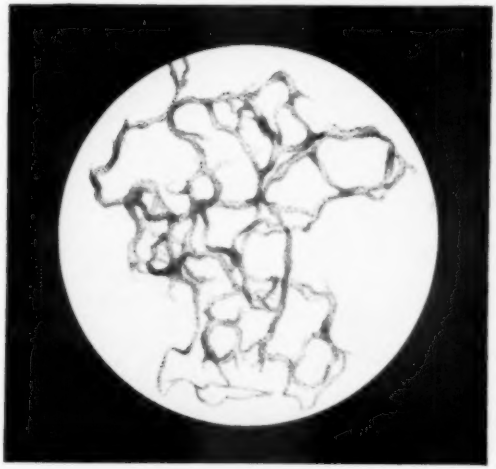

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ISONIAZID (ISONICOTINIC ACID HYDRAZIDE) IN THE TREATMENT OF MILIARY AND MENINGEAL TUBERCULOSIS[1,2,3]

CHARLES M. CLARK,[4] DuMONT F. ELMENDORF, Jr., WILLIAM U. CAWTHON
CARL MUSCHENHEIM, AND WALSH McDERMOTT

(Received for publication July 18, 1952)

INTRODUCTION

The demonstration that isoniazid has a high degree of antituberculous activity *in vitro* and in experimentally infected animals was made independently and apparently simultaneously in three different laboratories (1, 2, 3). In addition, in a fourth laboratory (4) the preliminary observations from one of the three laboratories (2) were considerably extended to include detailed studies in two additional animal species. Pharmacologic studies of isoniazid and several derivatives were made in a number of appropriate animal species by two groups of investigators (5, 6). The first investigation of derivatives of the isoniazid series in humans was made by Robitzek and Selikoff and was the basis for a preliminary report (7, 8). An independent study of the pharmacodynamics and antituberculous activity of isoniazid in humans was started by the present investigators in November, 1951. Observations on the absorption and distribution of isoniazid and its antituberculous activity in the body fluids of man made in the study have been previously reported (9). A third independent study of the use of isoniazid in man has also been published (10).

The present report is concerned with the investigation of the use of isoniazid in children and adults with acute generalized hematogenous tuberculosis (miliary) and tuberculous meningitis. The investigation has been conducted as a joint

[1] From The New York Hospital-Cornell Medical Center and the Indian Medical Service.

[2] Parts of this study were presented before the American Epidemiological Society, Pittsburgh, Pennsylvania, April 4, 1952; the Association of American Physicians, Atlantic City, New Jersey, May 6, 1952; the annual meeting of the National Tuberculosis Association, Boston, Massachusetts, May 26, 1952; and the Empire and Commonwealth Health Conference, London, England, July 9, 1952.

[3] This study was supported in part by grants from the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service; a generous grant from the Navajo Tribal Council; grants from The Lederle Laboratories Division, American Cyanamid Company, Pearl River; Charles Pfizer & Company, Brooklyn; and E. R. Squibb & Sons, New York.

[4] Senior Assistant Surgeon, U. S. P. H. S. (Indian Service).

enterprise at the New York Hospital-Cornell Medical Center and on the Navajo and Hopi reservation in Arizona.

PLAN OF INVESTIGATION

Patients: The patients treated were children or adults with acute generalized hematogenous (miliary) tuberculosis, tuberculous meningitis, or a combination of both forms of tuberculous disease.

The diagnosis of *miliary* tuberculosis was based on the presence of the triad: an acute febrile illness, the characteristic disseminated densities in roentgenograms of the chest, and the demonstration of acid-fast bacilli on microscopic examination of body discharges or of *M. tuberculosis* by culture.

The diagnosis of *meningeal* tuberculosis was established by culturing *M. tuberculosis* from the cerebrospinal fluid or by the demonstration of the cerebrospinal fluid findings of nonpurulent meningitis with a low concentration of sugar (less than 35 mg. per 100 ml.) in a patient with a bacteriologically proved lesion of tuberculosis elsewhere.

The majority of the patients were treated at the Western Navajo Hospital, Tuba City, Arizona, or at The New York Hospital. Four patients were treated at the Sage Memorial Presbyterian Mission Hospital, Ganado, Arizona,[5] and two at the Navajo Medical Center, Fort Defiance, Arizona.[5] With but few exceptions, all of the patients were examined repeatedly by all of the investigators.

Observations: The type and timing of the clinical, bacteriologic, and roentgenographic observations made of the course of the miliary and meningeal infections during isoniazid administration are similar to those listed in previous reports from this laboratory (11).

General management: During the acute illness, all of the patients were maintained on bed rest with the exception of bathroom privileges in a few instances. In some of the meningitis cases after clinical remission had been obtained, a certain degree of ambulatory activity within the hospital wards was permitted.

Drug: Isoniazid was supplied in scored tablets or as a highly purified crystalline powder incorporated in capsules containing 10 to 25 milligrams.[6]

Dosage regimen: With relatively few exceptions, all patients received approximately 10 mg. of isoniazid per kg. of body weight daily throughout the first week's administration. Irrespective of the course at that time, the dose was usually lowered to between 7 and 5 mg. per kg. daily thereafter. In no instances has therapy been discontinued, and at present it is planned to continue the chemotherapy of each patient for a total period of twelve months.

Chemical assay of isoniazid in body fluids: In some of the patients, determination of the concentration of isoniazid in the various body fluids was made by the

[5] The writers are particularly indebted to Dr. W. D. Spining, Director of Sage Memorial Hospital, and Dr. David Mock, of the Navajo Medical Center, and Dr. J. B. Chastain, Keams Canyon, for providing us with the opportunity to treat these patients.

[6] Generously supplied by E. R. Squibb & Sons as Nydrazid.

technique of Kelly and Poet (12). The results of these determinations were in general agreement with those previously published from this laboratory (9).

OBSERVATIONS

A total of 25 patients with acute generalized hematogenous (miliary) or meningeal tuberculosis have been treated with isoniazid. Five patients in the group (all with meningitis) received streptomycin in addition to the isoniazid and will be considered separately. The remaining 20 patients received only isoniazid during the period of study although a small number, all with meningitis, had previously received various forms of antituberculous chemotherapy (*see* below).

TABLE 1

DEFERVESCENCE AND ROENTGENOGRAPHIC CHANGE IN UNCOMPLICATED ACUTE MILIARY TUBERCULOSIS TREATED WITH ISONIAZID

PATIENT	DATE ISONIAZID BEGUN	INTERVAL TO DEFERVESCENCE	INTERVAL TO ROENTGENOGRAPHIC IMPROVEMENT	INTERVAL TO COMPLETE ROENTGENOGRAPHIC DISAPPEARANCE OF MILIARY NODULATION
		days	*days*	*days*
P. T.	1-30-52	23	26	130
J. S.	2- 4-52	7	14	51
E. D.	3-11-52	34	14	73
L. J.	3-12-52	43	88	117
J. L. B.	3-26-52	28	69	111
E. Di.	4-30-52	14	40	>76
I. J.	5- 1-52	47	55	>75
K. Y. M.	5-31-52	49	>45	>45
D. S.	6-10-52	13	35	>35
K. N.	6-17-52	26	>28	>28

Isoniazid was the sole antituberculous drug used in any of the patients with uncomplicated[7] miliary tuberculosis save in one patient who had received a single intramuscular injection of 1.0 gm. of streptomycin.

The series of 20 patients was composed of 10 with uncomplicated miliary tuberculosis, 4 with miliary tuberculosis complicated by meningitis, and 6 with meningitis alone.

The pertinent details concerning these 10 uncomplicated miliary patients, including the duration of therapy in the individual cases, are presented in table 1.

Generalized Hematogenous (Miliary) Tuberculosis

Ten patients with uncomplicated miliary tuberculosis have been treated with isoniazid. In every instance the institution of isoniazid therapy has been

[7] The term "uncomplicated miliary tuberculosis" is used to designate instances of miliary tuberculosis without meningitis.

followed by remission of all clinical evidences of infection and by regression or complete disappearance of the densities previously visible on chest roentgenograms.

Four additional patients with miliary tuberculosis complicated by meningitis have been treated with isoniazid alone. Two of these patients died of their meningitis after four and six days of therapy, respectively. The third patient was irrational and occasionally comatose, exhibiting positive Brudzinski's and Kernig's signs. She improved dramatically within two weeks to the extent that she was mentally alert and able to eat and walk unattended. The physical signs of meningitis had also disappeared. She is currently in her seventh week of treatment and has maintained her early clinical improvement. The fourth patient had only bacteriologic (culture) evidence of involvement of the central nervous system by *M. tuberculosis* and has experienced a favorable course identical with that of the patients with uncomplicated miliary tuberculosis.

Thus, of the entire series of 14 patients with miliary tuberculosis who have been treated solely with isoniazid, 12 are alive with apparent remission of their infections and 2 have died, both from meningitis during the first week of isoniazid therapy. (Only one other patient with miliary tuberculosis has received isoniazid. In this case the miliary disease was overshadowed by a severe tuberculous meningitis which has been treated successfully with a variety of measures.

No evidences of meningitis have appeared in the patients who were free of this complication when isoniazid therapy was instituted.

Duration of therapy: In 7 of the 14 miliary cases, including the 2 fatalities, isoniazid therapy was started between January 30 and March 12, 1952. Therapy was started in the remaining 7 cases between March 26 and June 17, 1952.[8]

Measurable Components of Infection

Defervescence: The speed with which complete defervescence occurred varied considerably between extremes of 7 and 49 days. In general, during the first week of isoniazid administration the total number of hours of fever above 38°C. was considerably less than it was prior to chemotherapy. Nevertheless, occasional rises of temperature to as high as 39°C. occurred as late as the twenty-first day of therapy in 2 of the 12 patients. In only one of the 12 was complete defervescence obtained by the end of the first week of isoniazid administration.

Representative examples of the most rapid and least rapid instances of defervescence may be seen in figures 1 and 2. Moreover, in figure 3 may be seen the weekly mean of the daily high temperatures of all of the 8 patients during the first seven weeks of treatment compared with a group of patients treated with streptomycin alone in 1946 and 1947.

Roentgenographic changes: The administration of isoniazid was followed in every instance by an impressive degree of clearing of the disseminated densities

[8] As of September 15, 1952, the status of the 12 surviving patients with miliary tuberculosis remains unchanged from the description in this article except that further roentgenographic clearing has occurred in the more recently treated cases (also see Addendum).

previously visible on chest roentgenograms. The speed with which this occurred varied considerably among the 12 patients. The shortest period of therapy in which definite roentgenographic improvement occurred was 14 days; the longest period without definite evidence of favorable roentgenographic change was 88 days.

Complete disappearance of the disseminated densities visible in the roentgenograms occurred in all of 5 patients who have received 130 days of isoniazid therapy. The shortest period required for complete disappearance of the dis-

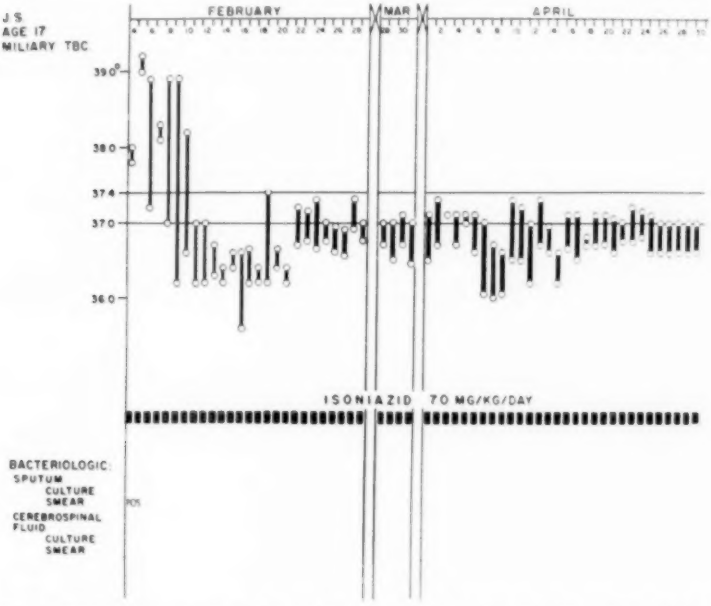


FIG. 1. Temperature chart of the patient (J. S.) who exhibited the most rapid defervescence. The patient has remained afebrile to date (July 15, 1952).

seminated densities was 51 days (figure 4). In contrast, one patient (figure 5) required 130 days for complete disappearance of miliary nodules. In some instances the disseminated densities on the roentgenogram were associated with a pneumonic component of either primary complex type or with cavity. In such instances, as may be seen in figures 6 and 7, complete disappearance of the disseminated densities occurred while the pneumonic component persisted but was obviously regressing

Bacteriologic course: M. tuberculosis was cultured from the sputum and gastric washings of 6 of the 12 surviving patients, and acid-fast bacilli were demonstrable on microscopic examination of sputum and gastric contents of all of the

remainder. Cultures of sputum and gastric washings from all of the 12 patients were obtained between July 15 and July 30. *M. tuberculosis* was isolated in one of the patients who had received 15 weeks of therapy at this point. In this instance, however, the miliary tuberculosis had been associated with a pneumonic component which presumably represented the source of the tubercle bacilli. In the 2 patients who had received less than 5 weeks of therapy, *M. tuberculosis* was isolated in both. Without exception, therefore, the reversal of infectiousness represented a reflection of the degree of roentgenographic change.

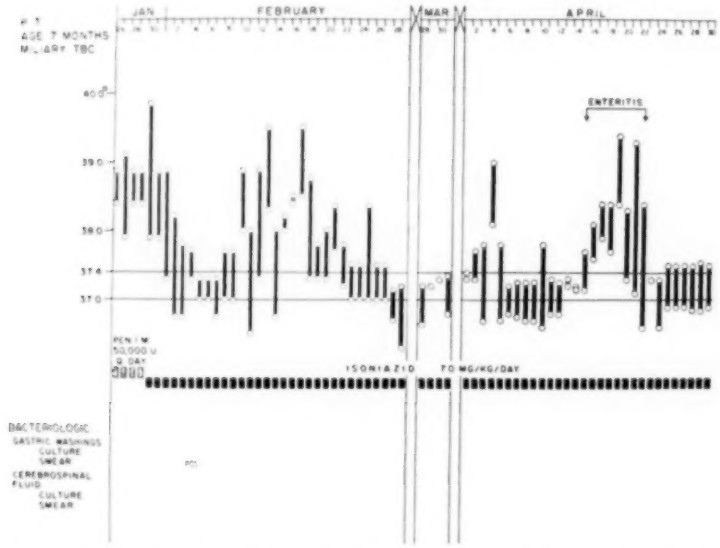


FIG. 2. Temperature chart of the patient (P. T.) who exhibited the slowest defervescence. No explanation was found for the initial diphasic pattern. The patient has remained afebrile to date (July 15, 1952).

In no instance were bacilli isolated from patients in whom no roentgenographic abnormalities were demonstrable on examination of the chest.

Symptomatic improvement: Despite variation in the speed with which defervescence occurred, the general appearance of the patients during the first week of isoniazid therapy was more satisfactory than prior to the institution of chemotherapy. The principal evidences of this satisfactory appearance consisted of increased alertness in previously somnolent patients, ability to feed themselves of small children and infants who had previously required gavage, and increased appetite in the adults. The increase of appetite appeared to be clearly related to the reduction of the toxicity of the disease as measured by the diminution or disappearance of fever. During the first 2 months of treatment, weight

gains which ranged from 0.7 to 11 kg. occurred in the 12 patients. The least amount of weight gain, 1.6 pounds, was in an 11-pound infant. In every instance, however, the increase in weight represented for the most part restoration of weight previously lost during the acute illness. Once defervescence had occurred and had been maintained, increments of weight gain were less impressive.

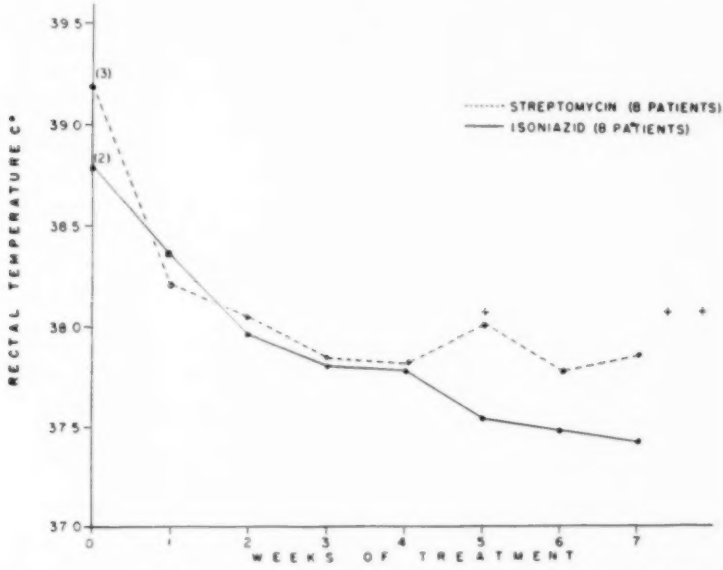


FIG. 3. Comparison of the temperature courses of initially uncomplicated miliary tuberculosis treated with streptomycin alone and with isoniazid alone.[1]

(*1*) Each point plotted represents the average of 56 values, i.e., the weekly average of the daily high temperature for the 8 patients in each group.

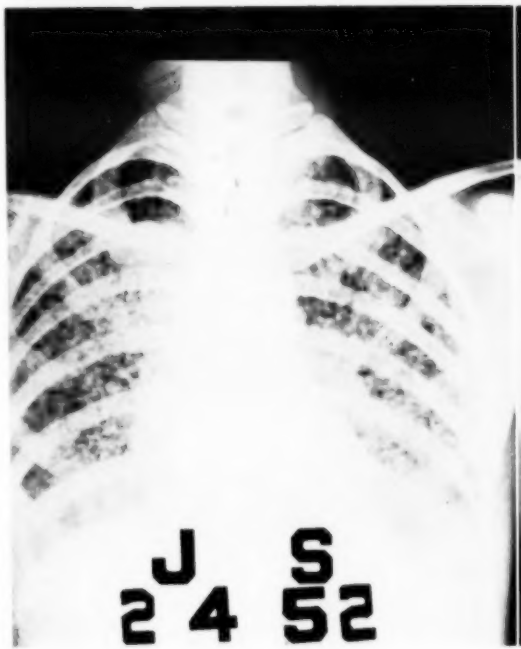
(*2*) Pretreatment average of 26 temperatures only.

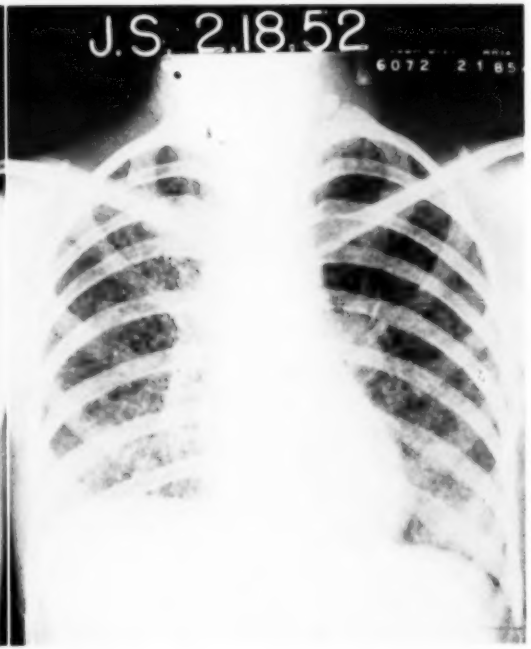
(*3*) Pretreatment average of 36 temperatures only.

* Onset of tuberculous meningitis in the streptomycin-treated group only at thirty-fifth, fifty-second, and fifty-fourth days of treatment.

Comparison of effects with those produced by streptomycin: An attempt was made to compare three indices of change: defervescence, roentgenographic change, reversal of infectiousness in the patients who received isoniazid and in the group treated by the present investigators in 1946 with 3 gm. daily of streptomycin.

In comparisons with the speed of defervescence, only streptomycin cases in which no meningitis was present at the start of therapy were included. A comparison of the weekly mean of the daily high temperatures in the first 7 weeks of therapy in the two groups may be seen in figure 3. There were no significant

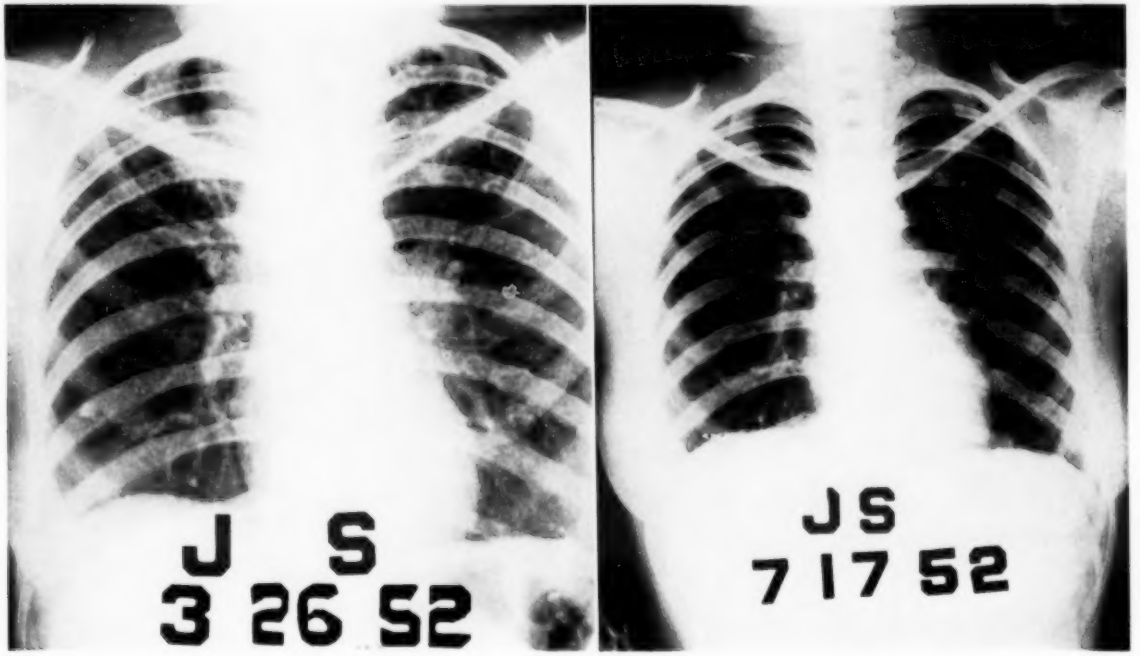




Case J. S.

FIG. 4A. Pretreatment roentgenogram

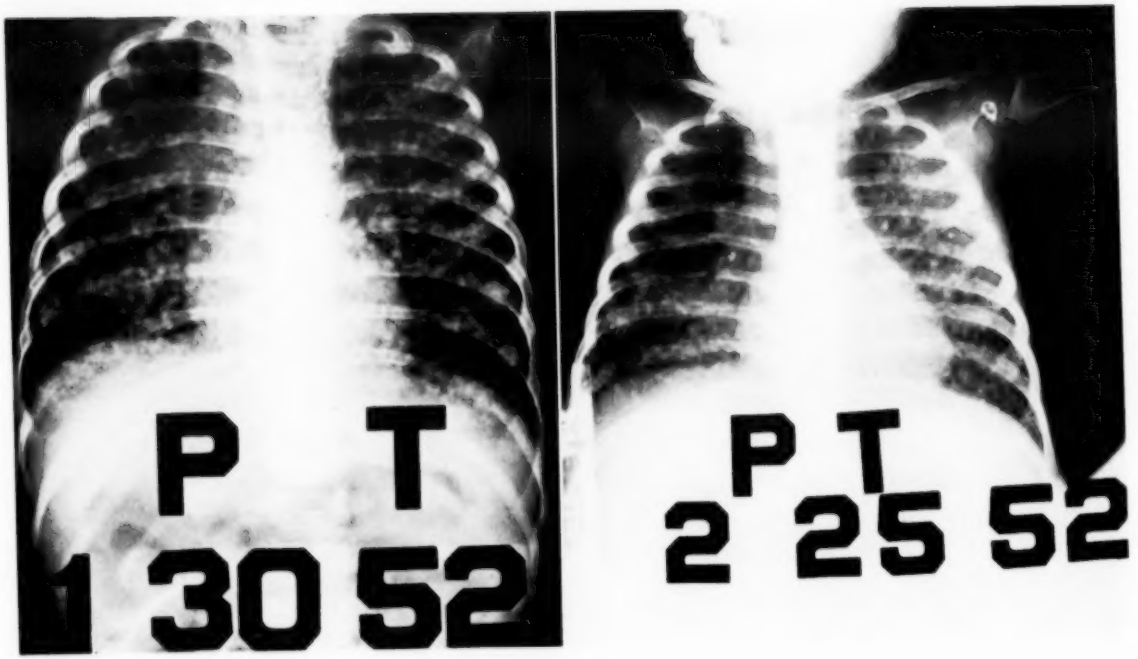
FIG. 4B. First roentgenographic evidence of improvement (14 days)



Case J. S.

FIG. 4C. First roentgenogram showing disappearance of miliary nodulation (51 days)

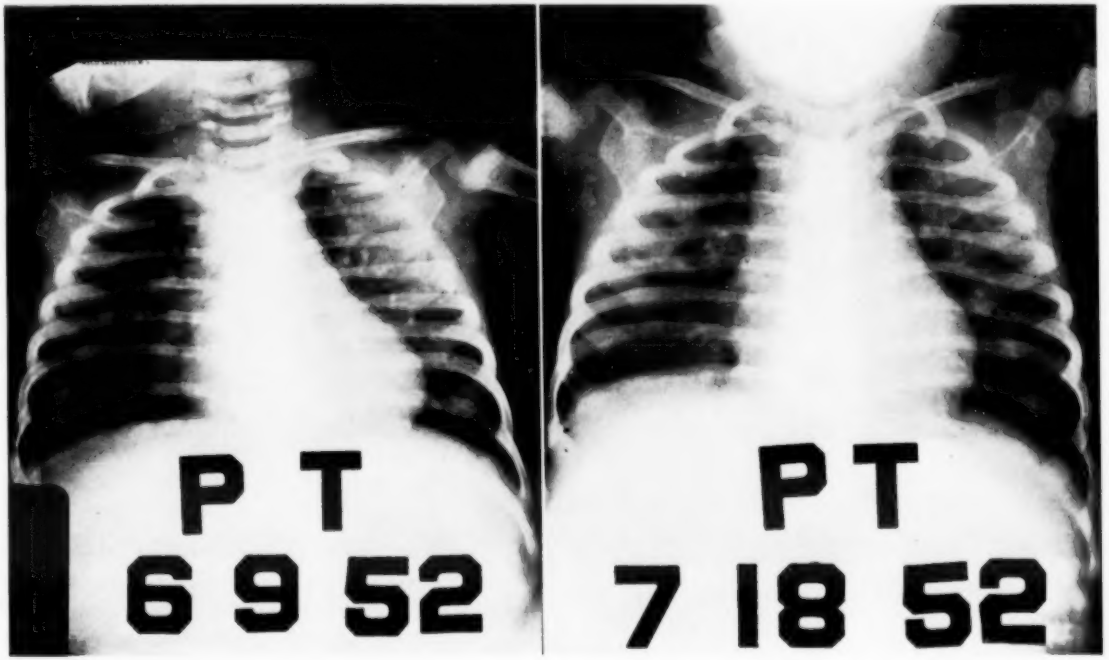
FIG. 4D. Most recent roentgenogram



Case P. T.

FIG. 5A. Pretreatment roentgenogram

FIG. 5B. First roentgenographic evidence of improvement (26 days)

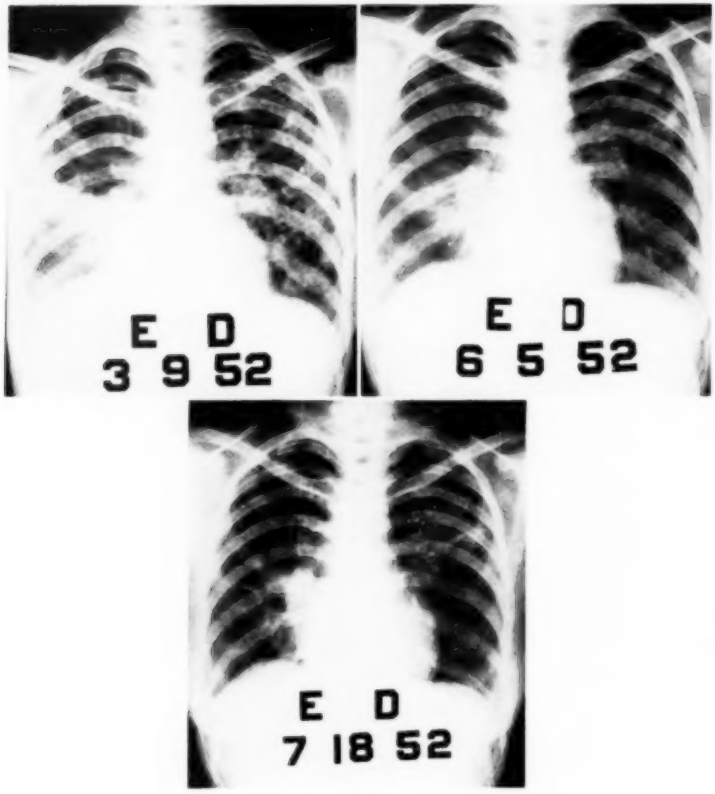


Case P. T.

FIG. 5C. First roentgenogram showing disappearance of miliary nodulation (130 days)

FIG. 5D. Most recent roentgenogram

differences in the speed with which complete defervescence occurred nor in the extremes of the shortest and longest periods of therapy required before the afebrile state was attained.

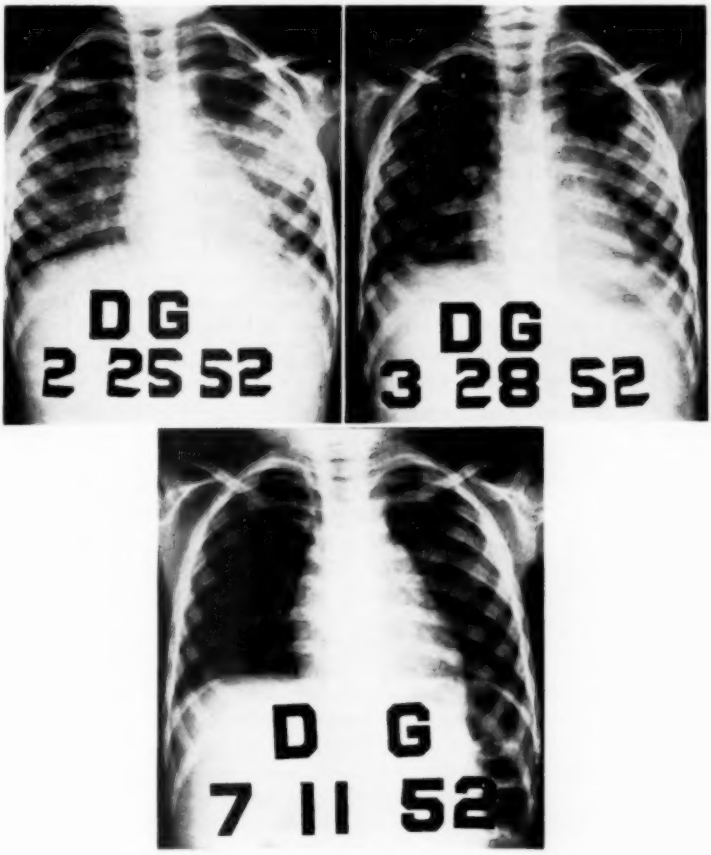


Case E. D.

FIG. 6. (Upper left) Pretreatment roentgenogram. (Upper right) Roentgenogram showing disappearance of the miliary nodulation which occurred while the pneumonic component improved, although it still persists. (Bottom) Roentgenogram showing further improvement of the pneumonic component.

In comparisons of roentgenographic changes, streptomycin-treated patients with miliary tuberculosis with or without meningitis were included. Roentgenograms from a total of 8 patients were available for study. The earliest definite regression of the disseminated roentgenographic densities was visible on the twenty-ninth day of streptomycin therapy. In 2 patients some evidence of

scattered densities persisted for so long as 130 days of chemotherapy. In 7 of the 8 streptomycin-treated patients, an impressive degree of roentgenographic clearing occurred by the ninety-ninth day and complete disappearance of the densities



Case D. G.

FIG. 7. (Upper left) Pretreatment roentgenogram. (Upper right) Roentgenogram showing disappearance of the miliary nodulation which occurred while the pneumonic component improved, although it still persists. (Bottom) Roentgenogram showing further improvement of the pneumonic component.

was observed in 6 by the one hundred and fifty-seventh day of streptomycin therapy. The last-named values, 99 and 157 days of therapy, are essentially the same as the corresponding values of 88 and 130 days of the isoniazid-treated series.

All of the 8 patients with miliary tuberculosis who received streptomycin were excreting tubercle bacilli at the time therapy was started. Four months after the start of streptomycin therapy (the corresponding period studied with the isoniazid-treated series), *M. tuberculosis* could be isolated from only 3 of the group. These results are to be compared with the value of 2 of 9 for the corresponding time period in the isoniazid-treated series.

Meningitis

As mentioned above, 4 of the 14 patients with miliary tuberculosis also had meningitis. In 2 of the 4, the meningitis proved fatal within less than one week of high-dose isoniazid therapy (10 mg. per kg. daily). The third patient described above was critically ill with tuberculous meningitis, but improved dramatically within two weeks. Her cerebrospinal fluid cells, protein, and sugar have improved steadily but are not yet normal at the seventh week of treatment. In the fourth patient, the only evidence of meningeal involvement consisted of the presence of *M. tuberculosis* in the cerebrospinal fluid during the acute stage of the miliary tuberculosis. The cerebrospinal fluid cultures promptly became negative during therapy. During the ensuing four months, the patient has shown no evidences of meningitis either clinically or on examination of the cerebrospinal fluid. As a consequence of this experience, the only patients who have been treated for meningitis with isoniazid alone have been patients with tuberculous meningitis who have failed to respond to other forms of therapy.

Exclusive of the 4 meningeal cases which complicated miliary tuberculosis, a total of 6 patients with tuberculous meningitis have been treated with isoniazid alone. One of these patients developed meningitis as a consequence of tuberculosis of one of the vertebra, and died during the first week of isoniazid therapy. Thus, 3 of the 10 patients with meningeal tuberculosis who received only isoniazid died during the first week of therapy.

The remaining 5 patients with tuberculous meningitis who received only isoniazid had previously received various amounts of streptomycin by both the intramuscular and intrathecal routes. As a consequence, at the time isoniazid therapy was started, they presented one of two situations. Two of the patients (B. Z. and E. B.) had been steadily deteriorating, whereas 3 presented only cerebrospinal fluid abnormalities as the sole evidence of meningitis.

One of the 2 patients whose condition had been deteriorating (B. Z.) was in status epilepticus at the time isoniazid therapy was started. Her convulsions were controlled by barbiturates, but she never regained consciousness and has remained for a period of six months in a state of stupor or coma. During this time, abnormal components of her cerebrospinal fluid have changed toward the normal pattern, as may be seen in table 2 (B. Z.) The other patient whose disease had been progressing despite streptomycin therapy will be described in more detai .

The patient was a 10-year-old Navajo girl who was admitted to the hospital in November, 1951, with the characteristic clinical findings of a nonpurulent meningitis. Cerebrospinal fluid examination revealed 480 cells, concentration of total protein of 150 mg. per 100 ml., and a concentration of sugar of 18 per 100 ml. No acid-fast organisms were seen on micro-

scopic examination of the sediment, nor was *M. tuberculosis* cultured at this time. Nevertheless, the patient was treated on a presumptive diagnosis of tuberculous meningitis and received 0.75 gm. of streptomycin intramuscularly each day and 60 mg. by the intrathecal route at forty-eight-hour intervals for a total period of 90 days.

During the three-month period of streptomycin therapy, the patient's condition became steadily worse. Consciousness was maintained, but the patient was frequently disoriented and all four extremities developed marked increased resistance to passive movement and were contracted in flexion. Any attempts to move the patient or to extend the extremities by passive motion produced loud cries and every appearance of considerable pain. The patient's daily temperature elevation at this time ranged between 1° and 2°C.

At the time isoniazid therapy was started, the patient's clinical description was as stated above. Her cerebrospinal fluid examination revealed 222 cells; total protein, 194 mg. per 100 ml.; and 22 mg. of sugar per 100 ml. In addition, *M. tuberculosis* was cultured from the cerebrospinal fluid immediately prior to the institution of isoniazid.

Isoniazid therapy, 10 mg. per kg. daily, was started on January 28, 1952, and has been continued. The dosage was lowered to 7 mg. per kg. daily at the end of the third day of treatment. Following the institution of isoniazid therapy, no dramatic changes occurred, but improvement gradually became perceptible. By the end of 21 days, the patient had recovered the use of her arms sufficiently to feed herself; and by 60 days her legs had improved sufficiently to permit her to walk with assistance. Ninety days after the start of therapy, she appeared mentally well, was afebrile, had full use of her arms, and was walking about the ward with a gait abnormal only because of weakness. The cerebrospinal fluid abnormalities present at the start of isoniazid therapy gradually receded, as may be seen in table 2 (E.B.).

The course of the 3 patients with tuberculous meningitis who showed only abnormal cerebrospinal fluids at the start of isoniazid therapy has remained unaltered (clinically). All were asymptomatic at the start of therapy and have remained so. In every instance abnormalities present in the cerebrospinal fluid at the start of isoniazid therapy gradually receded, and, with one exception (B. Z.), essentially normal values for the various components were all present by the twenty-second week of therapy. The previous streptomycin therapy and the course of the cerebrospinal fluid abnormalities in these patients are presented in table 2.

Tuberculous Meningitis Treated with Isoniazid Orally and Streptomycin by the Intramuscular Route

As a consequence of the deaths which occurred during the use of isoniazid alone, all subsequent patients with tuberculous meningitis received streptomycin by the intramuscular route as well as isoniazid. A total of 5 patients have been cerebrospinal fluid as demonstrated by culture), the first 7 to 23 days of therapy consisted solely of isoniazid. In both instances some degree of improvement was noted, but no dramatic change in the course of meningitis occurred. Accordingly, intramuscular streptomycin was added to the regimen without appreciably changing the speed of the steady improvement. In addition, 3 of these 5 patients subsequently received streptomycin by the intrathecal route as well; 2 remain treated solely by isoniazid and streptomycin intramuscularly.

One of the 5 patients in the isoniazid-streptomycin group died on the sixth day of therapy. The remaining 4 patients all are showing slow improvement after 31 to 43 days of therapy.

No comparative therapeutic inferences are permissible from this group of patients with tuberculous meningitis treated with isoniazid and streptomycin.

TABLE 2

THE COURSE OF CEREBROSPINAL FLUID FINDINGS IN PATIENTS WITH TUBERCULOUS MENINGITIS TREATED WITH ISONIAZID

PATIENT AND DATES OF STREPTOMYCIN PAS THERAPY*	PRECEDING STREPTOMYCIN AND PAS				CEREBROSPINAL FLUID ON ISONIAZID THERAPY (10 mg./kg./day for one week, then 7 mg./kg./day, divided in two doses per day)			
	Streptomycin Dosage†	PAS Dosage by Mouth	Cerebrospinal Fluid Four Weeks Before Discontinuing Streptomycin and PAS		0 Weeks	by 4 Weeks	by 12 Weeks	by 22 Weeks
		gm./kg./day						
B. Z., 5-7-51 to 1-26-52	IM, 60 mg./kg./day IT, 60 mg. q 2 days	0.2	Cells	1,070	230	22	18	38
			Protein	200	150	88	84	104
			Sugar	34	37	68	53	43
E. B., 11-28-51 to 1-28-52	IM, 60 mg./kg./day IT, 35 mg. q 2 days	0.2	Cells	200	222	33	15	14
			Protein	—	194	95	55	28
			Sugar	45	22	49	57	62
R. M., 1-6-51 to 1-26-52	IM, 60 mg./kg./day IT, 50 mg. q 2 days	0.2	Cells	13	71	14	24	7
			Protein	118	128	66	53	50
			Sugar	51	53	62	54	64
E. J., 5-31-51 to 1-26-52	IM, 60 mg./kg./day IT, 50 mg. q 2 days	0.2	Cells	24	22	10	0	5
			Protein	34	33	26	21	22
			Sugar	53	59	49	60	49
R. J., 8-7-51 to 1-26-52	IM, 20 mg./kg./day IT, 50 mg. q 2 days	0.2	Cells	102	44	60	49	20
			Protein	61	62	49	43	26
			Sugar	29	45	43	55	47

* Isoniazid begun on the day streptomycin and PAS were discontinued.

† IM = Intramuscular.
IT = Intrathecal.

DISCUSSION

On the basis of the evidence presented, it appears that isoniazid exerts a high degree of antituberculous activity in humans. The evidence consists of the demonstration in uncomplicated miliary tuberculosis of uniform disappearance of clinical illness, regression of roentgenographic abnormalities, and a high incidence of reversal of infectiousness following the institution of isoniazid therapy. Supportive evidence of antituberculous activity is provided by the results obtained in certain of the meningeal infections treated with isoniazid.

In general, there are three ways in which the activity of a drug against human tuberculosis may be established: observations on the abolition of clinical illness in patients critically ill with pulmonary tuberculosis; studies in miliary tuberculosis; and comparison of the drug with an established form of chemotherapy

by a chance-selection method in chronic pulmonary tuberculosis. All three methods have advantages and disadvantages, and it is believed that all three should be used in proper sequence in the evaluation of a new drug.

Miliary tuberculosis represents the ideal naturally occurring situation for the evaluation of an antimicrobial drug in three important particulars: The course of the untreated infection is generally uniform and predictable with a fatal outcome. Interpretation of results is not complicated by the presence of large areas of necrotic lung. A change in the ratio of drug-susceptible to drug-resistant parasites *of sufficient magnitude to neutralize drug activity* will be almost invariably followed by speedy relapse.

To be sure, subacute forms of hematogenous tuberculosis exist and may produce miliary densities on the chest roentgenogram. Such subacute infections are not necessarily fatal. Nevertheless, when the chest roentgenographic findings of miliary tuberculosis are associated with an acutely progressive highly febrile illness with tubercle bacilli in the sputum, survival for more than a few months is an extremely rare occurrence. In effect, therefore, it is only that portion of hematogenous infections which presents the acute syndrome of miliary tuberculosis which has a predictable course. In the present study, all of the 14 infections represented instances of this acute form of hematogenous disease. Except for streptomycin, none of the previously available antituberculous drugs is effective in controlling this syndrome of acute miliary tuberculosis. Hence, the uniformly favorable course of the 10 uncomplicated infections during isoniazid therapy represents impressive evidence of the high degree of antituberculous activity of the drug.

Once impressive drug activity has been demonstrated, a major objective is to determine at what point in time drug activity becomes significantly neutralized by the presence of drug-resistant tubercle bacilli. It cannot be too strongly emphasized that this is a most complicated problem and one not susceptible to easy answers.

There are two principal difficulties in the solution of this question. First, it has been established for both streptomycin (13) and isoniazid (14, 15), that drug-resistant variants may be present within the host before any drug therapy has been administered. Second, the ability of the host to control pulmonary tuberculosis may be so great that even a short period of artificial control of an acute illness (e.g., four to six weeks) may be all that is necessary to provide a remission extending far beyond that period. In the latter circumstances, it would be possible to witness drug effect in terms of the abolition of acute illness and mistakenly to attribute improvement in the months thereafter to the continued administration of the drug. This might easily be true even though the drug had lost all effectiveness after the first or second month of therapy.

Contrariwise, within narrow limits, an apparent increase in the amount of pulmonary tuberculosis during chemotherapy cannot always be regarded as evidence of neutralization of drug activity. The imperfections of roentgenography are such that a particular lesion may remain hidden from view on several

successive examinations, only to become visible thereafter as an apparently new lesion. Changes in cavity size of a considerable degree may also occur without representing any real changes in the drug-parasite-host relationships. Indeed, it is even conceivable that essentially mechanical events, such as hemorrhage or the endogenous aspiration of a fragment of bacillus-rich caseous material, might result in the implantation of drug-susceptible tubercle bacilli in circumstances which peculiarly favor the evolution of a detectable lesion despite continued antimicrobial therapy.

Thus, in pulmonary tuberculosis, relapse is by no means an inevitable consequence of the neutralization of drug activity on the one hand, nor does its occurrence necessarily indicate loss of drug activity on the other hand.

By contrast, in miliary tuberculosis, in the only chemotherapeutic situation in which information is available, i.e., streptomycin therapy, the termination of drug activity because of drug-resistant microorganisms is followed almost immediately by a return of the complete acute miliary syndrome. This phenomenon was observed in the present clinic in 5 patients with acute miliary tuberculosis who were treated solely by streptomycin (11). In 4 of the 5 cases, streptomycin-susceptible tubercle bacilli were isolated in preponderance before therapy; and in all 5, the institution of streptomycin therapy (3.0 gm. daily in eight divided doses) was followed by substantial or complete regression of all evidences of disease. A description of the phenomenon is as follows:

> During the first 30 to 90 days of streptomycin treatment, there was a hitherto unprecedented regression of the lesions which presumably represented an effect of the antimicrobial therapy. Despite the continued administration of the drug, however, there was a reappearance of all of the clinical, roentgenographic, and bacteriologic evidences of miliary tuberculosis in 4 instances, and a similar relapse seventeen days after the cessation of therapy in a fifth case. During relapse, a fresh dissemination of organisms occurred under therapy in at least 2 patients, as shown by the appearance of bacteremia and choroidal tubercles. Unlike the course displayed in the early phases of treatment, all 4 of the second bouts of miliary tuberculosis were completely uninfluenced by streptomycin and steadily progressed to a fatal termination.[9] At post-mortem examination, bacilli obtained from the lesions were as resistant to streptomycin *in vitro* as the organisms cultured during life. The appearance of relapse coincided in time with the appearance of tubercle bacilli which were highly resistant to the action of streptomycin *in vitro*. Conversely, drug-resistant bacilli were never obtained from the 7 patients who recovered from meningeal, miliary, or other forms of generalized hematogenous infections.

It is of interest to scrutinize the time of the appearance of relapse in these streptomycin-treated patients against the background of the duration of therapy without relapse in the patients who have received isoniazid. In the 4 streptomycin-treated patients in whom relapse occurred despite uninterrupted therapy, the phenomenon was first detectable on treatment days 20, 52, 66, and 85, respectively. By contrast, on July 15, 1952, 6 of the 12 surviving isoniazid-treated patients had received 90 to 268 days of therapy without relapse, and

[9] The fifth patient also died but had received very little streptomycin for the relapse.

the remainder had received 30 to 90 days of therapy with no evidence of relapse.[10]

Either of two inferences is permissible from the above comparison: Isoniazid maintains its high level of antituberculous activity in miliary tuberculosis for an appreciably longer period than is the case with streptomycin; isoniazid exerts sufficiently greater activity than streptomycin during the early therapy of miliary tuberculosis so that the host is better able to assert and maintain final control of the infection. On the basis of the present evidence, it is not possible to determine which of the two possible inferences is the correct one.

The information to be derived from the observations in the meningeal cases is considerably less precise than that obtained from the miliary infections. Nevertheless, the results are of importance.

Meningeal tuberculosis, when untreated, has a uniformly predictable outcome and in this respect is comparable with miliary tuberculosis as a situation for the evaluation of antimicrobial drugs. In all other respects, however, meningeal tuberculosis presents features which make therapeutic interpretation difficult to an extent even greater than is true with pulmonary disease. The clinical and cerebrospinal fluid findings throughout a group of patients with tuberculous meningitis may be essentially the same, yet the underlying lesions in the individual cases may show wide variation. Comparable clinical illnesses may be produced, on the one hand, by a single minute tuberculous focus or a diffuse process; and, on the other, by one or more tuberculomas several centimeters in diameter. Moreover, the localizing reaction of the host in the form of a cellular exudate at the base of the brain may itself become the disease. When sufficiently organized, the exudate can act either as a tumor and produce hydrocephalus with resultant brain damage or it can be the site of gross caseation with all that implies in terms of the persistence of viable parasites. Finally, gross sterile infarcts of the brain substance may be produced by tuberculous involvement of arterial walls.

It is obvious that an antimicrobial drug, however powerful, would have very limited effectiveness were certain of these lesions present, whereas a high degree of drug activity might be noticeable in the early diffuse forms of meningeal tuberculosis. It may be stated, therefore, that *success* in the antimicrobial therapy of tuberculous meningitis constitutes definite evidence of drug activity, but therapeutic *failure* may or may not mean that the drug in question lacks important antituberculous activity.

In the present study, one definite success in the treatment of tuberculous meningitis was observed in patient *E. B.* Moreover, in patient *A. A.*, who was eventually treated with both streptomycin and isoniazid, the latter drug was used alone for the first 22 days after the diagnosis, during which interval she showed steady improvement. Patient *D. G.* represents the third instance in which evidence of isoniazid activity within the central nervous system was obtained. In this instance the sole manifestation of meningeal involvement was

[10] No evidence of relapse in any of the 12 patients has appeared by September 15, 1952.

the presence of *M. tuberculosis* in the cerebrospinal fluid when isoniazid therapy was started. The cerebrospinal fluid became sterile soon after the start of therapy and has remained so; it has also remained normal in all other respects during the 4.5-month observation period (July 15, 1952[11]). With the use of streptomycin by the intramuscular route in this same type of meningeal involvement, it has been the experience in this and other clinics (11, 16, 17) that the cerebrospinal fluid usually becomes progressively abnormal and clinical meningitis eventually appears.

From the observations in the 3 meningeal cases cited and the previous studies on the easy transfer of biologically active isoniazid into the central nervous system, it appears that this drug may be of considerable value in the treatment of tuberculous meningitis. It is unlikely that isoniazid will ever be used as the sole chemotherapy in meningitis because of the diverse nature of the lesions and the possibility of drug-neutralization by significant changes in the ratio of drug-susceptible to drug-resistant bacilli. Nevertheless, there is reason to hope that the use of isoniazid orally and streptomycin by the intramuscular route might permit the elimination of the intrathecal use of streptomycin without sacrificing the maximally attainable antimicrobial activity within the central nervous system.

No therapeutic inferences are permissible at this time from the observations on the other surviving patients with tuberculous meningitis who have received isoniazid alone or in association with streptomycin (5 cases). The 4 patients who died despite isoniazid therapy serve as convincing evidence that there are definite limits to the effectiveness of the drug in this form of tuberculosis.

Acute miliary tuberculosis not only provides a situation in which the presence and duration of antituberculous drug activity can be measured; the acute disease also presents a situation in which crude quantitative comparisons between two drugs can be made. The principal criteria[12] by which such comparisons can be made are: rate of defervescence, rate of roentgenographic clearing, incidence of meningitis first appearing after the start of therapy, and incidence of relapse due to drug-resistant *M. tuberculosis*. In the present study, such comparisons were made with the corresponding observations previously made in this laboratory on the influence of streptomycin on miliary tuberculosis (11). A comparison of the two studies has the disadvantage that they were not conducted concurrently. Nevertheless, the syndrome of acute miliary tuberculosis, as defined above, was presumably the same in the two different time periods and the observations were organized and the data interpreted by the same persons (C. M. and W. McD.) in association with other investigators.

With regard to rates of defervescence and roentgenographic clearing, no differences between the two drugs could be demonstrated. The question of relapse due to drug-resistant tubercle bacilli has been discussed above. Within

[11] Situation unchanged as of September 15, 1952.

[12] Reversal of infectiousness is not a helpful criterion in such comparisons in miliary tuberculosis. In many instances the sole bacteriologic proof of miliary tuberculosis consists of the isolation of *M. tuberculosis* from an excised lymph node or from bone marrow.

a time period in which 4 of 8 streptomycin-treated infections relapsed, there have been no relapses in 7 isoniazid-treated cases.[13] It is not believed that the isoniazid series has been followed as a whole for a sufficient period to make the complete absence of meningitis meaningful. To be sure, no instances of meningitis have occurred in the 10 patients since the start of therapy, whereas 4 instances of meningitis occurred within 79 days of the start of streptomycin among the 8 patients in the latter series. Nevertheless, at present (July 15, 1952) only 7 of the isoniazid group have been treated for such a period.[14]

To sum up this portion of the discussion: A comparison of the uniformly satisfactory results in the isoniazid-treated uncomplicated miliary cases with the results previously obtained in this clinic with streptomycin permits the inference that the antituberculous activity of isoniazid in man is equivalent, and probably slightly the superior, of the antituberculous activity of streptomycin.[15]

Without question, the major problem presented by tuberculous infection of man is pulmonary disease and not the miliary type of involvement. The question immediately arises, therefore, of the proper relation of the information on antimicrobial activity which can be derived from miliary tuberculosis to the problems presented by pulmonary tuberculosis. There is no reason to doubt that the same high degree of antituberculous activity of isoniazid which can be so easily detected in miliary tuberculosis is also operative in patients with pulmonary tuberculosis. In the latter situation, however, the presence of substantial areas of necrosis may interfere considerably with both the detection of drug activity and the end results attainable with any form of antimicrobial therapy.

Isoniazid and streptomycin both rank high as antimicrobial drugs in terms of such attributes as ability to intefere with vital activities of a parasite, distribution and maintenance of activity within mammalian hosts, and reasonable toleration on long-continued administration. To be sure, isoniazid has certain advantages over streptomycin in that the former is readily transferred into the central nervous system and into monocytes (18, 19). As antimicrobial drugs, however, both substances compare very favorably indeed with the antimicrobial drugs used in the treatment of other infections. Thus, the limitations on what may be accomplished by isoniazid and streptomycin in pulmonary tuberculosis arise not from any special lack of effectiveness on their part as drugs but from the nature of pulmonary tuberculosis with its destructive component.

[13] No relapse has been observed as of September 15, 1952, in any of the 12 patients.

[14] No instances of meningitis have occurred in any of these 12 patients as of September 15, 1952. Hence, the difference between the isoniazid and the streptomycin series with respect to the development of meningitis after the start of therapy is considered to be significant.

[15] As para-aminosalicylic acid (PAS) was not used in the previous studies of miliary tuberculosis in this clinic, it is not possible at this time to make inferences concerning the respective antituberculous activities of isoniazid and streptomycin used in conjunction with PAS. Such an inference should soon be permissible, however, from a comparison of the incidence of meningitis in the previously uncomplicated miliary cases of the present study and similar series treated elsewhere with streptomycin and PAS.

The action of both drugs, so far as is known, is limited to a direct effect upon the metabolic activities of a parasite. It is established, moreover, that these drugs are not eradicative for *M. tuberculosis* in mammalian hosts, as indeed few drugs are for any infection. Hence, the action of isoniazid or streptomycin in tuberculosis must necessarily be confined to interference with the metabolic activities of *M. tuberculosis* in such a way as to influence the production of clinical illness and to prevent further destruction of tissue. In situations such as miliary tuberculosis, in which the infection becomes clinically recognizable before substantial focalized destruction of tissue has occurred, antimicrobial therapy may be as highly effective as in most other bacterial and rickettsial infections. Contrariwise, in situations such as pulmonary tuberculosis, in which a considerable amount of destruction has usually taken place before the presence of disease is recognized, the accomplishments of antimicrobial therapy may be correspondingly limited.

The general question of the influences of necrosis on the effectiveness of antimicrobial agents is one of outstanding importance but is not strictly germane to consideration of the present study. Nevertheless, one aspect of the question does merit comment. It is highly probable that the differences between the miliary lesions and those of chronic pulmonary disease present different situations in terms of the emergence to predominance of drug-resistant tubercle bacilli.

It has been established that isoniazid-resistant tubercle bacilli can be isolated with ease from tuberculous patients, irrespective of whether the patient has ever received the drug (14, 15). Whether these drug-resistant variants have the same pathogenicity as drug-susceptible strains has not yet been established. Moreover, the "back-mutation" rate of *M. tuberculosis* from the isoniazid-resistant to the isoniazid-susceptible type has not been defined. If it be assumed, however, that at least some strains of isoniazid-resistant tubercle bacilli possess full pathogenicity and a low "back-mutation" rate, it would follow that continuation of isoniazid *in the presence of unhealed lesions* would lead eventually to preponderance of drug-resistant bacilli with consequent neutralization of drug activity. Precisely this set of conditions is provided by certain chronic pulmonary lesions, notably those with a cavitary component. An opportunity is thus provided for the maintenance of a continually multiplying microbial population although the microbial census may remain essentially stable.

In miliary tuberculosis, in contrast, the necrotic component of the individual lesions is usually minute. Consequently, it should provide no great obstacle either to the maintenance of full drug activity or to the host mechanisms for control of the parasites and promotion of healing. Hence, the microbial census should be steadily diminishing during therapy even though a certain amount of multiplication may continue. In these circumstances the chances for the emergence to predominance of the drug-resistant descendants of drug-susceptible microbes is correspondingly limited.

For these reasons, the fact that microbial population shifts of significance in terms of the neutralization of drug activity have failed to occur in the patients of the present study by no means implies that such shifts might not occur in

patients with chronic destructive lesions. It is permissible to infer, as mentioned previously, that in miliary tuberculosis the emergence to predominance of drug-resistant tubercle bacilli occurs at an earlier period with streptomycin than with isoniazid. Nevertheless, as certain lesions of pulmonary tuberculosis may be so chronic, it seems reasonable in such circumstances to use two or more antimicrobial drugs concurrently in the hope that the emergence to predominance of drug-resistant tubercle bacilli may be postponed or prevented.

SUMMARY

An investigation has been made of the influence of isoniazid on the course of acute miliary tuberculosis in 14 patients, 4 of whom also had meningitis. An additional 11 patients with tuberculous meningitis (2 of whom also had miliary tuberculosis) received isoniazid together with, or subsequent to, streptomycin therapy.

Two deaths, both from meningitis, occurred in the 14 patients with acute miliary tuberculosis who were treated solely with isoniazid. Both the deaths occurred during the first week of therapy. In the remaining 12 of the isoniazid-treated acute miliary group, the institution of therapy was followed by a uniform disappearance of clinical illness, regression of reontgenographic abnormalities, and a high incidence of reversal of infectiousness.

A comparison of the uniformly satisfactory results in the isoniazid-treated uncomplicated miliary infections with the results previously obtained in this clinic with streptomycin permits the inference that the antituberculous activity of isoniazid in man is the equivalent, and probably slightly the superior, of the antituberculous activity of streptomycin.

Additional evidence of the antituberculous activity of isoniazid was provided by the results obtained in certain of the meningeal infections.

SUMARIO

La Isoniacida (Hidracida del Acido Isonicotínico) en el Tratamiento de la Granulia y la Tuberculosis Meníngea

En esta investigación estudióse el influjo de la isoniacida sobre la evolución de la tuberculosis miliar aguda (granulia) en 14 enfermos, 4 de los cuales tenían además meningitis. Otros 11 enfermos con meningitis tuberculosa (2 de los cuales tenían además granulia) recibieron isoniacida junto con, o después de, la estreptomiconiterapia.

Hubo 2 muertes, todas de meningitis, entre los 14 enfermos granúlicos que fueron tratados solamente con isoniacida. Todos esos fallecimientos ocurrieron durante la primera semana de terapéutica. En los otros 12 enfermos del grupo granúlico tratado con isoniacida, la aplicación de dicho tratamiento fué seguida de desaparición uniforme de la enfermedad clínica, regressión de las anomalías radiográficas y alta incidencia de viraje de la infecciosidad.

Una comparación de los resultados invariablemente satisfactorios obtenidos en las infecciones granúlicas sin complicaciones tratadas con isoniacida con los obtenidos anteriormente en la misma clínica con estreptomicina permite inferir que la actividad antituberculosa de la isoniacida en el hombre es equivalente, y con toda probabilidad ligeramente superior, a la de la estreptomicina.

Más pruebas de la actividad antituberculosa de la isoniacida las aportaron los resultados conseguidos en algunas de las infecciones meníngeas.

RESUME

L'isoniazide (l'acide isonicotiane hydrazine) dans le traitement de la tuberculose miliare et la meningite tuberculeuse

Les recherches etaient faites sur l'influence de l'isoniazid sur quatorze cas de tuberculose miliaire aigue dont quatre avaient fait la méningite. Onze autres cas de méningite (dont deux avait tuberculose miliaire) ont été traité par l'isoniazid avec on ultérieurement de la streptomycine.

Il y avait deux cas de morts de méningite parmi les quatorze (tuberculose miliarie aigue) qui ont été traité seulement par l'isoniazid. Tous les décès se passait pendant la premiere semaine du traitement. Dans les douze autres de ce groupe de miliaire aigue traité par l'isoniazid, le traitement était suivi par un disparition uniform des symptoms cliniques de la maladie, regression des abnormalities roentgenographiques et un fréquence assez notable de cessation de l'infectivité.

Une comparison de ces résultats de l'isoniazid dans les miliaires non-compliqués avec les résultats obtenu dans notre clinique par la streptomycine nous laisse déduire que l'activité antituberculeux de l'isoniazid dans l'homme est égale et peut être un peu superieur a l'activité antituberculeux de la streptomycine.

Un preuve supplementaire de l'activité antituberculeux de l'isoniazid était apporté par les résultats obtenus dans certain des infections méningites.

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Addendum

Since the above manuscript was set in type, *M. tuberculosis* was cultured from the otherwise normal cerebrospinal fluid of patient I. J. (table 1) on treatment days zero and 84. (Therapy was started May 1, 1952.) In patient E. D., a submental lymph node ruptured on approximately the one-hundredth day of treatment and drained purulent material which yielded *M. tuberculosis* on culture. There has been no evidence of relapse of the miliary infection of this patient as of treatemnt day 188.

STUDIES ON THE METABOLISM OF VIRULENT AND AVIRULENT MYCOBACTERIA[1, 2]

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(Received for publication April 28, 1952)

INTRODUCTION

The major difference between *Mycobacterium tuberculosis* var. *hominis* H37Rv and its avirulent variant, H37Ra, is the ability of the former to grow and reproduce in the tissues (1, 2). Since, in the final analysis, this must be a reflection of differences in metabolism, studies were initiated to determine, if possible, the metabolic capacities of the two strains and to correlate these with the presence or absence of the ability to produce disease.

Of the two methods available for measuring the response of the tubercle bacillus to various metabolites, effect on respiration or on growth rate, the latter was considered to furnish more informative data for the purpose of the study. The reason for this choice is the fact that the substances which stimulate respiration markedly do not always comparably effect the growth of the organisms (3, 4).

For estimation of growth rate, the minimal inoculum technique (5) was considered the method of choice since it avoids the necessity of adding Tween 80[3], the presence of which may introduce indeterminable or unknown alterations in metabolism (6, 7, 8, 9). Moreover, the virulence of tubercle bacilli may be reduced by growth in Tween-albumin medium (10).

The use of a simple, chemically defined medium in which the tubercle bacillus can synthesize its essential cellular constituents and in which virulence, as judged by median survival time in mice, is maintained more nearly satisfies the requirements of this experiment to quantitate a comparative study of the potential metabolism of a virulent and avirulent strain of *Mycobacterium tuberculosis* var. *hominis*. The method has the further advantage that growth is measured in the early logarithmic phase, and readings are made at a time when measured inocula reach approximately the same concentration of organisms. Therefore, a minimum number of variables are introduced. Such variables as the effect of reduction in nutrients, production of metabolites, or alteration in pH, which may become limiting factors in themselves, are avoided.

This investigation presents the results of a study of the method, applied to the further elucidation of the effect of serum and of a number of vitamins on the growth of virulent and avirulent strains of tubercle bacilli.

[1] From the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

[2] This investigation was supported (in part) by a research grant from the National Institutes of Health, Public Health Service.

[3] Proprietary name of the polyoxyethylene derivative of Sorbitan monooleate, obtained from Atlas Powder Co., Wilmington, Delaware.

MATERIALS AND METHODS

Microorganisms: The following mammalian strains of *Mycobacterium tuberculosis* were utilized: 2 strains, the virulent strain H37Rv and the avirulent variant H37Ra, which have been maintained in this laboratory; 5 avirulent variants (11, 12) of virulent strains,[4] JH16Ra, JH6Ra, H4Ra, R1Ra, and another subculture of H37Ra. The bovine strain, BCG,[5] was also included.

Maintenance of cultures: Stock cultures were maintained as a surface growth on a modified Proskauer and Beck medium (P & B) by routine subculture of two- to three-day-old cultures. In the case of the avirulent variant H37Ra, which grew more compactly and from which it was difficult to remove a small inoculum, subcultures were made from five- to seven-day-old cultures. This not only ensured a ready supply of cultures of the proper age which grew well in P & B medium at the time needed for experimental work, but also afforded tubercle bacilli of a comparatively similar physiologic age. Moreover, virulence of the H37Rv strain is best maintained when grown on the surface of a chemically defined medium (1).

Media: The modified Proskauer and Beck medium previously described by Youmans (13) served as a basal medium to which substances to be tested were added. Substances tested included the vitamins riboflavin,[6] vitamin B_{12},[6] choline,[6] pantothenic acid,[6] biotin,[6] pyridoxine,[6] thiamin,[6] *i*-inositol,[6] niacin,[6] niacinamide,[6] and coenzyme I;[6] the bovine plasma fractions II,[7] III,[7] IV-3,4,[7] IV-1,[7] V,[7] crystalline bovine albumin,[7] proantithrombin,[8] fibrinolysin[8] (fraction III-3), antifibrinolysin,[8] prothrombin[8] (fraction III-2), fibrinogen[8] (fraction I), and bovine plasma ultrafiltrate.[9] These substances were added to the basal medium in the desired concentration, and the pH was adjusted to 7.0 by the addition of sodium hydroxide or hydrochloric acid. Sterilization was accomplished by filtration through Berkefeld (N) or sintered glass filters (UF). If several concentrations of a substance were to be tested, dilutions from a concentration prepared as described were made aseptically into accurately measured quantities of sterile P & B medium.

Bovine serum, obtained fresh every two weeks, was sterilized by filtration through Berkefeld (N) or sintered glass filters (UF), and added aseptically to the P & B basal medium in the designated concentration.

Cleaning of glassware: In the first half of the experiment, glassware was cleaned in dichromate cleaning solution, rinsed ten to twelve times in tap water, followed by three rinses in distilled water. Because of the large amount of glassware involved, a comparison was made in duplicate experiments between test tubes washed in dichromate solution and in a detergent. Since no difference in results was noted in glassware washed by either method, in the latter half of the experiment the glassware was washed in detergent,[10, 11] rinsed ten to fifteen times in hot, followed by cold, water and finally three times in distilled water. All glassware was sterilized at 20 pounds for thirty minutes before use.

Preparation of inocula: Portions of eighteen- to twenty-one-day-old cultures were lifted with a 4 mm. loop, allowed to drain for a few seconds, and aseptically transferred to a sterile 200 by 25 mm. Pyrex tube containing glass beads, and ground by describing by hand a circle of approximately 4 inches in diameter approximately 150 times per minute. Ten milliliters of sterile 0.01 molar phosphate buffer were added, the suspension was thoroughly mixed and allowed to stand to permit large clumps to settle out. After thirty min-

[4] Obtained from the Trudeau Laboratory through the courtesy of Mr. W. Steenken, Jr.

[5] Furnished by Dr. Joseph D. Aronson from The Henry Phipps Institute.

[6] Obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio.

[7] Obtained from Armour and Company, Chicago, Illinois.

[8] Furnished through the courtesy of E. C. Loomis, of Parke, Davis & Company, Detroit, Michigan.

[9] Microbiological Association, Inc., Flemington, New Jersey.

[10] F. G. D. powder (without benchlophen), Kalusoff, Ltd., Springfield, Illinois.

[11] Marvarox, grade 74, Antiseptol Company, Chicago, Illinois.

utes the finely dispersed supernate was carefully pipetted off and standardized by centrifuging 1 ml. of the well-mixed suspension in a Hopkins vaccine centrifuge tube for twenty minutes at 1,000 r.p.m. The 0.01 ml. mark on the Hopkins tube arbitrarily was taken to represent 10 mg., wet weight, of tubercle bacilli. The exactness of this measurement is relatively unimportant. In the test proper, it is the accuracy of the dilutions relative to each other, rather than the absolute numbers of organisms present, which is important. Dilutions of the suspension in 0.01 molar phosphate buffer were made to contain 10^{-1} mg. per drop. From this, dilutions in phosphate buffer were made to contain 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} mg. per drop. A different pipette was used for each dilution. As can be noted, this method of preparing dilutions permitted the use of only a partially washed inoculum. Any growth factors carried over, however, would be markedly diluted out by the procedure.

Measurement of growth rate: Each substrate tested for effect on the growth rate of the tubercle bacillus was appropriately diluted in P & B medium and added aseptically in 5 ml. quantities to 32 Pyrex test tubes, 150 by 20 mm. in volume. This number of tubes was sufficient for duplicate tests, one set for H37Rv and one set for H37Ra; three tubes for each of 5 inocula and one tube for an uninoculated control. Similar sets containing the P & B medium and the same medium to which 10 per cent bovine serum was added were included each time to serve as controls. All media were incubated at 37°C. before inoculation to check for sterility. Three tubes of each set were then inoculated with each quantity of H37Ra and H37Rv. The drop method of inoculating was one previously described (14) and has been found by experience to give much less variation of inoculum in tubes inoculated with the same quantity than accurately measured quantities from a pipette. The inoculated tubes were incubated at 37°C. and examined daily at approximately the same time, and a record was kept when growth was first noted. Readings were made using a Quebec colony counter, which affords slight magnification and a constant background. When the day of appearance of growth is plotted against the logarithm of the numbers of tubercle bacilli inoculated, a straight line is obtained whose slope is a function of the generation time (5).

Method of calculating generation time: In order to obtain an unbiased estimate of the slope, b, the best straight line to fit each set of data was evaluated by the regression equation $Y = a + bX$. Since the data obtained showed very little deviation from regression, the reciprocal of the slope, b, can be accepted as the growth rate constant, K. The generation time is found by substituting this value of K into the formula:

$$G = \frac{\log 2}{K}.$$

Virulence of H37Ra and H37Rv: The virulence of H37Ra and H37Rv was assayed twice during the study, at the beginning and at the end of the experiments. This was done with H37Rv by injection of 1 mg.. wet weight, of organisms into the tail vein of 20 mice. With H37Ra, two sizes of inocula were used: 1 mg. into each of 20 mice and 7.5 mg. into an additional 20 mice. The results indicated that the level of virulence characteristic of each strain had been maintained during the course of these studies.

RESULTS

Critique of the method: Although sound in principle, the minimal inoculum technique has several limitations. In the first place, it is based on the assumption that microorganisms transferred to a suitable medium while in the logarithmic phase of growth continue to grow at a steady rate. This assumption is generally accepted in the case of other microorganisms, but no means has been available to establish it for the tubercle bacillus. Moreover, the growth rate is determined by an indirect method of measurement, and at the present time no direct way of validating the method has presented itself. Finally, the

method can be questioned because of the unavoidable subjective manner of reading results.

Nevertheless, some evidence of the validity of the theory and assumptions may be obtained in a statistical analysis of the data. Reference to figure 1 will show that what is being evaluated is the regression of Y, time of first appearance of growth in days, on X, the logarithm of the numbers of tubercle bacilli in the inoculum. The slope of the line, b, gives the change in Y in days per unit change in log X (size of inoculum) and is a function of the growth rate and directly proportional to the generation time. Therefore, if the organisms are growing at a steady rate, regardless of the size of the inoculum, a straight line should be obtained. Deviations from a linear relationship, then, would indicate lack

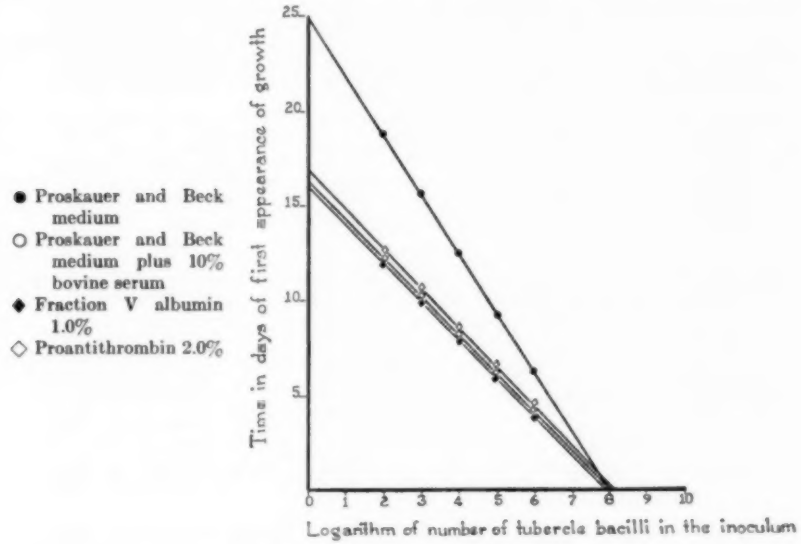
● Proskauer and Beck medium
○ Proskauer and Beck medium plus 10% bovine serum
◆ Fraction V albumin 1.0%
◇ Proantithrombin 2.0%
Time in days of first appearance of growth
25
20
15
10
5
0 1 2 3 4 5 6 7 8 9 10
Logarithm of number of tubercle bacilli in the inoculum

FIG. 1. Rate of growth of H37Rv in four media

of uniformity of growth rate for whatever reason, whether due to effect of substrate, technical errors, or to lack of soundness of the principle upon which the method is based.

Youmans and Youmans (15) have found by this method that H37Rv responds with an increased growth rate to the addition of serum and several plasma fractions, namely, proantithrombin, fibrinolysin, fractions IV-1 and IV-3, 4, fraction V, and crystalline albumin to the basal P & B medium. In the course of the present study, comparing the metabolism of the avirulent variant, H37Ra, with its virulent counterpart, H37Rv, the same substances have been tested. A statistical analysis was therefore made on the data obtained with H37Rv and H37Ra and compared with the results obtained by Youmans and Youmans (15).

Use of the regression, $Y = a + bX$, is based on the hypothesis that the variance of Y for any value of X is homogeneous. The data for eight individual experiments for H37Rv and H37Ra in P & B medium and in P & B with 10 per cent bovine serum added were

analyzed by Bartlett's test for homogenicity of variance (16 *a*). The results for each strain in each of the two media were pooled and the analysis of the data is given in table 1. The probability that the variance of Y for any value of X is the same is indicated by the value of chi-square, which in each case is much smaller than the value for the 0.01 level of probability. In P & B, to which 10 per cent bovine serum has been added, the data for H37Rv give remarkably reproducible straight lines; in 26 of 28 experiments, a straight line was obtained with a generation time of 14.4 hours. This observation was also made by Youmans and Youmans (5).

TABLE 1

HOMOGENEITY OF VARIANCE OF THE DEVIATIONS FROM REGRESSION OF H37Rv AND H37Ra IN PROSKAUER AND BECK MEDIUM AND IN PROSKAUER AND BECK MEDIUM CONTAINING 10 PER CENT BOVINE SERUM

STRAIN OF MYCOBACTERIUM TUBERCULOSIS	MEDIUM	NUMBER OF EXPERIMENTS POOLED	CHI-SQUARE	*P*
H37Rv	P & B	8	2.2666	>0.90
H37Rv	P & B + serum	8		
H37Ra	P & B	8	0.8399	>0.99
H37Ra	P & B + serum	8	5.4930	>0.60

TABLE 2

STANDARD ERROR OF ESTIMATE (Sy.x) OF TIME OF FIRST APPEARANCE OF GROWTH OF H37Ra AND H37Rv IN PROSKAUER AND BECK MEDIUM AND IN PROSKAUER AND BECK MEDIUM CONTAINING 10 PER CENT BOVINE SERUM

STRAIN OF MYCOBACTERIUM TUBERCULOSIS	MEDIUM	INDIVIDUAL EXPERIMENTS			POOLED ANALYSIS ON GROUPS	
		Number	Range of Sy.x in Days	Mean of Sy.x in Days	Number	Sy.x in Days
H37Rv	P & B	18	0.00–1.15	0.500	8 7	0.88 1.15
H37Rv	P & B + serum	28	0.00	0.000	8	0.00
H37Ra	P & B	22	0.00–0.87	0.311	8 7	0.92 0.67
H37Ra	P & B + serum	20	0.00–0.73	0.323	8 6	1.20 1.07

H37Rv and H37Ra have a generation time, as determined by this method, of a little less than twenty-four hours in P & B medium. Thus, in so far as frequency of observations is concerned, a daily reading differs little from thirty-minute readings on an organism which reproduces once in twenty minutes. The question has occurred, however, in regard to the error or insensitivity involved in reading the tests once daily. In table 2 the data for the two strains are presented, in P & B and in P & B to which 10 per cent bovine serum has been added, in terms of standard error of estimate (sy.x.), as determined by the method of linear regression (16 *b*). The range and mean value are given of sy.x for

the readings in the individual experiments, as well as for subgroups in which the data for the designated number of experiments were pooled and analyzed as one experiment.

The values on the individual experiments are an estimate of the error involved in reading the first appearance of growth, when variables such as the population of tubercle bacilli, media, pH, temperature, inoculum size, et cetera are the same. Comparison of these values with the larger error obtained when the data for a number of experiments are pooled and analyzed as a whole gives an indication of the unavoidable technical errors which may show variation from one experiment to another. The explanation for the marked reduction in sy.x. obtained for readings on H37Rv in serum medium is not known except for the obvious one that growth is more uniform. Greater ease of reading results in the serum medium can be eliminated as the cause of lessened variation since, as can be noted, H37Ra has approximately the same sy.x. in P & B with 10 per cent bovine serum as in P & B alone.

Another criticism which may be raised is the degree of bias which may enter into the subjective method of reading. A "blindfold" test was performed as follows: A test was

TABLE 3

GROWTH RATE OF H37Ra AND H37Rv IN PROSKAUER AND BECK MEDIUM. STATISTICAL SIGNIFICANCE OF THE DIFFERENCE OF THE MEAN GENERATION TIMES

	STRAIN OF MYCOBACTERIUM TUBERCULOSIS	
	H37Ra	H37Rv
Number of experiments	22	19
Mean generation time in hours	22.69	23.08
Standard deviation	±2.71	±2.82
Standard error	±0.58	±0.65
95% confidence limits	22.69 ± 1.20	23.08 ± 1.36
t*	0.465	
P	>0.50	

* A P value ≤ 0.01 requires that $t \geq 2.708$.

set up in P & B medium with 10 per cent bovine serum and was inoculated with H37Rv. An impartial observer was asked to rearrange the test tubes so that the individual reading the results would not be influenced by knowing in which tubes growth could first be expected. The results matched perfectly the order of inoculation, and the generation time conformed to previous tests using the same strain in the same medium.

Reproducibility of the controls: Since no other method is available at present for validating the results, some idea may be gained of the validity of the data by analyzing the degree of reproducibility of the generation times obtained from the data of the two strains of tubercle bacilli, H37Rv and H37Ra, grown in the control basal medium but tested at different times. In table 3, the mean generation time for 19 determinations of H37Rv and for 22 determinations of H37Ra when growing in P & B medium are given. The probability that the two strains have the same rate of growth in a chemically defined medium can hardly be questioned on the basis of these results. The high degree of reproducibility is also evident.

Effect of serum on H37Rv: The mean generation times for 19 determinations of the growth rate of H37Rv in P & B medium and for 28 determinations of the same organism in P & B containing 10 per cent bovine serum are shown in table 4. From these determinations performed over a period of a year, the gen-

TABLE 4

THE RATE OF GROWTH OF H37Rv IN PROSKAUER AND BECK MEDIUM AND IN PROSKAUER AND BECK MEDIUM CONTAINING 10 PER CENT BOVINE SERUM. STATISTICAL SIGNIFICANCE OF THE DIFFERENCE OF THE MEAN GENERATION TIMES

	MEDIUM	
	Proskauer and Beck	Proskauer and Beck with 10 Per Cent Bovine Serum
Number of experiments	19	28
Mean generation time in hours	23.08	14.55
Standard deviation	±2.82	±0.599
Standard error	±0.65	±0.115
95% confidence limits	23.08 ± 1.36	14.55 ± 0.236
t*	15.8	
P	$\ll 0.01$	

* A P value ≤ 0.01 requires that $t \geq 2.690$.

TABLE 5

THE RATE OF GROWTH OF H37Ra IN PROSKAUER AND BECK MEDIUM AND IN PROSKAUER AND BECK MEDIUM CONTAINING 10 PER CENT BOVINE SERUM. STATISTICAL SIGNIFICANCE OF THE DIFFERENCE OF THE MEAN GENERATION TIMES

	MEDIUM	
	Proskauer and Beck	Proskauer and Beck with 10 Per Cent Bovine Serum
Number of experiments	22	19
Mean generation time in hours	22.60	22.05
Standard deviation	±2.71	±2.05
Standard error	±0.58	±0.47
95% confidence limits	22.69 ± 1.20	22.00 ± 0.983
t*	0.853	
P	>0.40	

* A P value ≤ 0.01 requires that $t \geq 2.708$.

eration time of H37Rv in P & B medium can be accepted as 23.08 ± 2.82 hours. The generation time for H37Rv in 10 per cent bovine serum medium is 14.55 ± 0.599 hours. These results accord well with the results obtained by Youmans and Youmans (5). The value of t[12], it will be noted, for significance of the dif-

[12] The value for the significance of the difference of the means was obtained by the method for comparison of two groups (16 *c*).

ference of the mean generation times is high, indicating a highly significant difference in response to the two media and suggesting the presence in the serum of a stimulatory substance for the H37Rv strain of *Mycobacterium tuberculosis*.

Effect of serum on H37Ra: Experiments were conducted to determine whether the avirulent variant H37Ra was stimulated also in the presence of serum. In table 5 are given the results obtained in 22 determinations of H37Ra in P & B medium and in 19 determinations in P & B medium, to which 10 per cent bovine serum was added. The likelihood that serum differentiates H37Ra into two

TABLE 6

EFFECT OF BOVINE PLASMA FRACTIONS ON THE GROWTH RATE OF H37Rv. SIGNIFICANCE OF THE DIFFERENCE OF THE MEAN GENERATION TIME IN THE FRACTIONS COMPARED TO THE MEAN GENERATION TIME OF H37Rv IN PROSKAUER AND BECK MEDIUM

FRACTION	CONCENTRATION	NUMBER OF EXPERIMENTS	MEAN GENERATION TIME IN HOURS	t	P
	per cent				
Proantithrombin	2.0	6	14.90	6.932	$\ll 0.01$
V albumin	1.0	5	14.40	6.958	$\ll 0.01$
Crystalline albumin	1.0	2	17.70	2.721	<0.02
Antifibrinolysin	0.25*	3	18.75	2.705	<0.02
	0.125				
II	1.00*	3	20.19	1.710	>0.10
	0.25				
III-1	1.0	2	19.22	1.873	>0.05
IV-3, 4	1.0	1	19.44	†	
IV-1	1.0*	2	22.04	0.248	>0.50
	0.5				
Bovine plasma ultrafiltrate	10.0	1	24.72	†	
Prothrombin (III-2)	0.5*	2	23.19	0.053	$\gg 0.50$
	0.25				
Fibrinolysin (III-3)	0.5	2	27.47	2.120	<0.05
Fibrinogen (I)	0.25	2	28.28	2.500	<0.05
Control in Proskauer and Beck medium	—	19	23.08 ± 2.82		

* The generation time was essentially the same in both concentrations.

† Group comparisons of mean differences require a minimum of two determinations.

populations is extremely small as evidenced by the t value for significance of the difference of the means.

Effect of plasma fractions: Consistent with the stimulatory action of serum on H37Rv, as noted above, Youmans and Youmans (15) found that several plasma fractions also stimulated the growth of H37Rv. Of the fractions tested, they found that proantithrombin gave a generation time equal to that found in serum. It was also found that with this fraction alone the response was similar to that of serum, in that within certain concentrations it was proportional to the amount of serum or proantithrombin added.

The results obtained in the present experiment with the plasma fraction are

presented in table 6. With the exception of fractions IV-1, IV-3,4, and fibrinolysin, the results are similar, within the experimental error of the method, to those obtained by Youmans and Youmans (15). Fractions IV-1 and IV-3,4 showed no significant difference in growth rate, and fibrinolysin increased the generation time over that in the chemically defined medium. These three fractions were repeated a number of times, but the results remained consistently the same. It is probable that different lots of the same fraction may vary in purity since plasma fractions cannot be considered as pure chemical entities,

TABLE 7

EFFECT OF BOVINE PLASMA FRACTIONS ON THE GROWTH RATE OF H37Ra. SIGNIFICANCE OF THE DIFFERENCE OF THE MEAN GENERATION TIME IN THE FRACTIONS COMPARED TO THE MEAN GENERATION TIME OF H37Ra IN PROSKAUER AND BECK MEDIUM

FRACTION	CONCENTRATION	NUMBER OF EXPERIMENTS	MEAN GENERATION TIME IN HOURS	t	P
	per cent				
Proantithrombin	2.0	6	18.62	3.070	<0.01
V albumin	1.0	5	19.19	2.357	<0.05
Crystalline albumin	1.0	2	24.72	2.000	>0.05
Antifibrinolysin	0.25*	3	21.09	0.555	>0.50
	0.125				
II	1.00*	2	24.91	1.446	>0.10
	0.25				
III-1	1.00*	2	25.75	1.811	>0.05
	0.25				
IV-3, 4	0.25*	2	20.61	0.755	>0.40
	0.125				
IV-1	0.5*	2	26.25	2.432	<0.05
	0.25				
Bovine plasma ultrafiltrate	10.0	1	24.72	†	
Prothrombin (III-2)	0.25	1	23.76	†	
Fibrinolysin (III-3)	0.5	2	22.82	0.359	>0.50
Fibrinogen (I)	0.25	1	33.12	†	
Control in Proskauer and Beck medium	—	22	22.09 ± 2.71		

* The generation time was essentially the same in both concentrations.

† Group comparisons of mean differences require a minimum of two determinations.

and this may account for the results obtained with these fractions. On the other hand, in table 6, it can be seen that proantithrombin, fraction V, antifibrinolysin, and crystalline albumin all stimulated the growth of H37Rv. Fractions II, III-1, IV-1, IV-3,4, prothrombin, and bovine plasma ultrafiltrate had no significant effect on the generation time of H37Rv. Fibrinolysin and fibrinogen showed some inhibitory action. Not only was an increased generation time over the control observed, but an increase in inhibitory action was noted with increase in concentration of these fractions.

Since the addition of serum to the basal medium did not alter the generation time of H37Ra, the possibility was considered that serum contained an inhibi-

tory factor for this organism; therefore, the effect of separate plasma fractions on the growth of H37Ra was tested. The data are presented in table 7. No differential inhibition of the growth rate was shown by the plasma fractions tested; instead, it appears that the growth rate of H37Ra is not affected by several fractions, namely, crystalline albumin, antifibrinolysin, fractions II, III-1, IV-3,4, and bovine plasma ultrafiltrate. Moreover, whereas four fractions significantly stimulated the growth rate of H37Rv, only fraction V and proantithrombin effected a decrease in generation time of H37Ra. Figures 1 and 2 reveal graphically the growth rates of H37Ra and H37Rv in P & B and in the presence of serum, proantithrombin, and fraction V albumin.

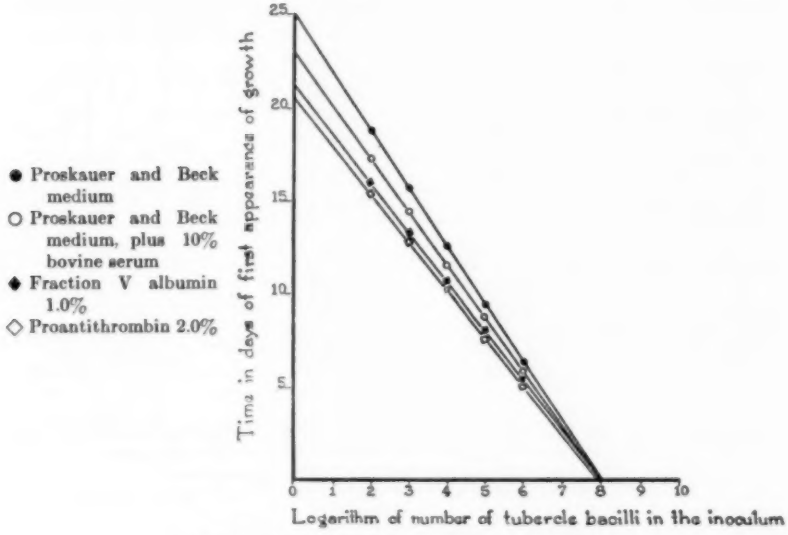
● Proskauer and Beck medium
○ Proskauer and Beck medium, plus 10% bovine serum
◆ Fraction V albumin 1.0%
◇ Proantithrombin 2.0%
Time in days of first appearance of growth
25
20
15
10
5
0 1 2 3 4 5 6 7 8 9 10
Logarithm of number of tubercle bacilli in the inoculum

FIG. 2. Rate of growth of H37Ra in four media

Effect of filtrates: The possibility was considered that the difference in response to serum and proantithrombin by the two strains might be explained by the ability of the virulent variant to alter the substrate or produce a substance which would stimulate H37Ra. Filtrates were prepared by seeding H37Rv and H37Ra in 1 mg. quantities each into plain P & B medium and into P & B medium to which 2.0 per cent proantithrombin had been added. After twenty-one days of incubation, the cultures were filtered through UF sintered glass filters, the pH adjusted to 7.0 with normal hydrochloric acid, and the supernate again filtered. A control flask of uninoculated 2.0 per cent proantithrombin was treated in the same manner. The filtrates were then added in various concentrations to the basal medium and the growth rates of each strain

of tubercle bacillus in each of the four filtrates was determined. The results, together with the controls, are presented in table 8.

The stimulation of H37Rv in both the proantithrombin culture filtrates was no more than would be expected on the basis of the probable proantithrombin content. The strain H37Ra, however, showed a slight decrease in generation

TABLE 8

GROWTH RATES OF H37Ra AND H37Rv IN FILTRATES

SUBSTRATE	CONCENTRATION	STRAIN OF MYCOBACTERIUM TUBERCULOSIS					
		H37Ra			H37Rv		
		Number of Experiments	Mean Generation Time	Standard Deviation	Number of Experiments	Mean Generation Time	Standard Deviation
	per cent						
Proantithrombin filtrate of H37Rv	25	4	16.14	±1.70	2	14.4	0.00
Proantithrombin filtrate of H37Ra	25	4	16.50	±1.33	2	14.4	0.00
Proantithrombin incubated and filtered	25	1	17.28				
Proantithrombin	2.0	6	18.62	±0.92	6	14.90	±0.90
Filtrate of H37Rv grown in P & B	25	2	22.92		1	18.48	
Filtrate of H37Ra grown in P & B	25	2	22.56		1	24.72	
Serum	10	1	21.84		1	14.40	
Proskauer and Beck medium	—	1	23.76		1	19.44	

TABLE 9

SIGNIFICANCE OF THE DIFFERENCE IN GENERATION TIME OF H37Ra GROWING IN THE PROANTITHROMBIN FILTRATES OF H37Ra AND OF H37Rv FROM THE GENERATION TIME IN 2 PER CENT PROANTITHROMBIN

SUBSTRATE	NUMBER OF DETERMINATIONS	MEAN GENERATION TIME	t	P
2 per cent proantithrombin	6	18.62 ± 0.92		
25 per cent filtrate of H37Rv grown in proantithrombin	4	16.14 ± 1.70	3.857	<0.01
25 per cent filtrate of H37Ra grown in proantithrombin	4	16.50 ± 1.33	3.620	<0.01

A P value ≤ 0.01 requires that $t \geq 3.355$.

time in the filtrate of both H37Rv and H37Ra. On the basis of four determinations, H37Ra gave a generation time of 16.14 hours in the proantithrombin filtrate of H37Rv and a generation time of 16.50 hours in the proantithrombin filtrate of H37Ra. Statistical analysis indicates a significant difference in generation time in both the filtrates over that in 2.0 per cent proantithrombin medium (table 9).

It should also be noted in table 8 that there is no significant difference in growth rates of either H37Ra or H37Rv in the P & B medium filtrates, as compared with the growth rate in P & B medium alone. This confirms the findings of Uyei (17) and Boissevain and Schultz (18), but differs from those of Borrel, Boez, and de Coulon (19) and Wangliang (20). Apparently neither strain produced in P & B basal medium within twenty-one days a substance which inhibits or stimulates the growth of either strain in the concentration employed.

Effect of serum, filtrates, and plasma fractions on avirulent dissociants of virulent strains: The response of other avirulent variants of virulent strains to serum, proantithrombin, fraction V, and to the filtrates was tested. For this purpose, 5 avirulent human strains and one bovine strain were used: JH6Ra, JH16Ra, H4Ra, R1Ra, a new subculture of strain H37Ra, and BCG. Another

TABLE 10

GENERATION TIME IN HOURS OF AVIRULENT VARIANTS OF HUMAN STRAINS AND ONE BOVINE STRAIN IN VARIOUS SUBSTRATES

SUBSTRATE ADDED TO PROSKAUER AND BECK MEDIUM	CONCENTRATION	STRAIN OF MYCOBACTERIUM TUBERCULOSIS							
		JH6Ra	R1Ra	JH16Ra	H4Ra	BCG	H37Ra*	H37Ra	H37Rv
	per cent								
None		No Growth	No Growth	18.48	No Growth	41.28	20.16	21.84	20.64
Bovine serum	10	31.20	26.64	18.24	15.84	15.84	18.96	21.24	14.40
Proantithrombin	2.0	21.84	23.28	14.40	25.68	17.76	15.84	18.48	14.40
	0.5	29.76	28.80	14.40	25.92	23.28	15.84	18.48	14.40
Fraction V albumin	1.0	23.28	21.84	17.06	22.56	19.24	18.24	17.88	14.40
Filtrate of H37Rv grown in proantithrombin	25.0	23.28	20.88	14.40	22.56	19.24	15.84	17.28	14.40
Filtrate of H37Ra grown in proantithrombin	25.0	23.28	19.44	—	—	—	17.06	17.28	14.40

* New strain of H37Ra.

subculture of strain H37Ra was tested in order to determine whether the response of the stock strain of this laboratory was due to independent variation. On receipt, the strains were transferred to the surface of P & B medium and maintained in the same manner as the H37Ra and H37Rv strains. At the time of the experiment, they had been subcultured nineteen times on the P & B medium.

Twenty-one-day-old cultures of the avirulent variants were used for the experiment with the exception of BCG. A tendency for the BCG pellicle to sink after approximately the twelfth to fourteenth day of growth on the surface of P & B medium was noted. In this instance, therefore, a 12-day-old culture was employed. Each strain in five dilutions was inoculated into plain P & B medium and into P & B medium to which 10 per cent bovine serum, 2.0 and 0.5 per cent proantithrombin, or 1.0 per cent fraction V albumin had been added. The new subculture of strain H37Ra, JH6Ra, and R1Ra were also inoculated into a 25 per cent concentration of the filtrates of H37Rv and H37Ra grown in 2.0 per cent proantithrombin. BCG, JH16Ra, and H4Ra were tested only in the filtrate of H37Rv grown in 2.0 per cent proantithrombin. The results are given in table 10.

TABLE 11

RATE OF GROWTH OF H37Ra AND H37Rv IN THE PRESENCE OF B VITAMINS AND COENZYME I

SUBSTRATE	ADDITION	CONCENTRATION IN γ PER ML.	STRAIN OF MYCOBACTERIUM TUBERCULOSIS	
			H37Ra	H37Rv
			Generation Time in Hours	Generation Time in Hours
Proskauer and Beck	Biotin	5×10^{-3}	20.30*	23.28
		5×10^{-4}	19.20*	23.28
		5×10^{-5}	19.80*	22.56
Proskauer and Beck + 10 per cent serum	Biotin	5×10^{-3}	20.88*	
		5×10^{-4}	20.40*	
		5×10^{-5}	20.88*	
Proskauer and Beck	Pyridoxine	10	23.28	24.72
		1.0	23.76	23.52
		0.1	21.84	23.76
		1×10^{-3}	24.72	23.28
Proskauer and Beck + 10 per cent serum	Pyridoxine	10	21.84	
		1.0	21.84	
		0.1	21.12	
		1×10^{-3}	24.62	
Proskauer and Beck	Thiamin	5.0	31.2†	†
		0.5	21.84	†
		5×10^{-3}	21.84	†
Proskauer and Beck + 10 per cent serum	Thiamin	5.0	23.28	
		0.5	23.28	
		5×10^{-3}	22.56	
Proskauer and Beck	*i*-Inositol	1,000	31.20	23.28
		100	26.64	†
		10	23.28	30.00
		0.1	23.28	28.80
Proskauer and Beck + 10 per cent serum	*i*-Inositol	1,000	27.60	
		100	25.68	
		10	23.28	
		0.1	22.56	
Proskauer and Beck	Niacin	5,000	—	—
		5	28.80	†
		5×10^{-3}	34.56	†
Proskauer and Beck + 10 per cent serum	Niacin	5,000	—	
		5	26.64	
		5×10^{-3}	25.68	

TABLE 11—*Continued*

SUBSTRATE	ADDITION	CONCENTRATION IN γ PER ML.	STRAIN OF MYCOBACTERIUM TUBERCULOSIS	
			H37Ra	H37Rv
			Generation Time in Hours	Generation Time in Hours
Proskauer and Beck	Niacinamide	5,000	—	—
		5	36.12†	†
		5×10^{-3}	36.12†	†
Proskauer and Beck + 10 per cent serum	Niacinamide	5,000	—	
		5	26.64	
		5×10^{-3}	23.76	
Proskauer and Beck	Coenzyme I	10	28.80	26.64
		0.1	26.64	24.72
		1×10^{-4}	23.28	23.28
Proskauer and Beck + 10 per cent serum	Coenzyme I	10	26.64	
		0.1	29.76	
		1×10^{-4}	27.60	

— No growth.
* Average of two determinations.
† Inhibitory to small inocula, 10^{-4} 10^{-4}.

With the exception of the new subculture of strain H37Ra and of strain JH16Ra, small inocula of the avirulent strains grew poorly as submerged growth in the plain P & B medium. Growth of these strains was made possible by the addition of serum, proantithrombin, or fraction V albumin and, therefore, the growth rate of these strains can be said to have been stimulated. Little more can be said concerning the significance of the differences in the various substrates. It may be noted, however, that the growth rate of the one strain which grew in the P & B medium, JH16Ra, seems not to be stimulated in the presence of serum and shows a decreased generation time in proantithrombin. JH6Ra and R1Ra showed greater stimulation of the growth rate by the addition of proantithrombin, fraction V, and filtrate than by serum. Furthermore, the growth response of JH6Ra, R1Ra, and BCG appears to be proportional to the concentrations of proantithrombin employed. Only H4Ra and BCG show a greater stimulation by serum than by the other substrates.

Effect of vitamins: Although the literature gives little encouragement to the belief that any of the known vitamins may be required by the tubercle bacillus, the possibility was considered that such a difference might exist between the virulent strains and their avirulent variants. The avirulent strains appear, in general, to be more metabolically deficient than their virulent counterparts (6, 21, 2). Certainly, the ability to synthesize their requirements for growth in a chemically defined medium appears to be less in the case of 3 of the strains tested above.

Ten of the vitamins and coenzyme I were tested for the effect on the growth

rate of H37Ra and H37Rv. Five of these, namely, riboflavin, vitamin B_{12}, choline, *p*-aminobenzoic acid, and pantothenic acid were found to have no effect. The results on the remainder are shown in table 11.

In general, the results (table 11) indicate that thiamin, *i*-inositol, niacin, niacinamide, and coenzyme I are inhibitory in high concentrations to both strains, but show a greater inhibitory effect on H37Rv in all concentrations tried except coenzyme I. Biotin and pyridoxine seem to have no effect on either strain. None of the growth factors affected the growth of H37Ra in serum except coenzyme I, which showed some inhibitory action.

Of interest is the apparent neutralization of some of the inhibitory effect of thiamin, niacin, and niacinamide on the growth rate of H37Ra by serum but absence of this effect with *i*-inositol. With coenzyme I, the presence of serum appears to increase the inhibition of the growth rate over that in P & B medium.

DISCUSSION

The present study has been concerned basically with the question whether metabolic differences between virulent and avirulent tubercle bacilli can be detected which might account for the observed difference in pathogenicity.

The rate of growth of H37Rv was found to be the same as that of H37Ra when grown in a simple chemically defined medium. In the basal medium to which bovine serum was added, the generation time of the avirulent variant was the same as in the chemically defined medium. This is in contrast to the marked stimulation of the growth rate of H37Rv by serum and may indicate a metabolic difference in two strains of common origin which differ in degree of pathogenicity.

It is of interest that the growth of strain H37Ra was stimulated in concentrations of 0.5 and 2.0 per cent proantithrombin and of 1.0 per cent fraction V albumin, but the rate of growth was less than the rate of growth of the virulent H37Rv in the same substrates. One might expect that, if both strains were being stimulated by the same substance, the effect would be the same. A number of interpretations are possible, but the mechanism whereby the same fractions stimulate both strains, but to a different degree, is not known. The finding, however, may indicate that two distinct stimulatory substances are involved.

The experimental data obtained on the effect on the growth of H37Ra of the filtrates of cultures of both the H37Rv and H37Ra strains when grown in 2.0 per cent proantithrombin for twenty-one days do not indicate that the strains alter the substrate in a differential manner. The degree of stimulation of the growth rate of H37Ra by both filtrates is the same although significantly greater than that produced by proantithrombin alone. If the difference in degree of stimulation by proantithrombin on the growth rate of the two strains of tubercle bacilli were explainable by a difference in rate of utilization, it might be expected that H37Rv would produce more of the metabolite and that the filtrate of H37Rv would then stimulate to a greater degree than the filtrate of H37Ra.

Proantithrombin and fraction V albumin both caused significantly greater stimulation of the growth rate of H37Ra than that produced by serum. This

suggests that an inhibitory factor for H37Ra may be present in whole serum. Whether the greater growth inhibitory effect of some of the plasma fractions for H37Ra can entirely account for this is not known.

It would appear also that the growth-stimulating function of serum is not solely protective. The plasma fraction showing the greatest stimulation is a globulin, which presumably would have a much lower ion-binding capacity than albumin. Youmans and Youmans (22) have also shown that the rate of growth of recently isolated virulent strains of *M. tuberculosis* var. *hominis* was stimulated more by proantithrombin, a globulin, than by crystalline albumin although these were tested in approximately similar molar concentrations.

In comparison with H37Ra, the growth rates of the other avirulent variants indicated perhaps a greater metabolic deficiency. Small inocula of three of the strains, JH6Ra, H4Ra, and R1Ra, were unable to grow in the basal medium alone. The BCG strain had a slower growth rate. The addition of serum and plasma fractions to the basal medium, however, enabled the growth of those strains which were unable to grow in basal medium alone. However, the rapid rate of growth shown by H37Rv in the presence of proantithrombin and in fraction V albumin was not obtained with any of the avirulent strains with the exception of strain JH16Ra. This strain had a generation time of 14.4 hours in proantithrombin medium and in medium to which had been added the filtrate of H37Rv grown in proantithrombin. The results obtained with this strain were not comparable to those obtained with H37Rv in other respects, however, since the growth rate in the basal medium and in serum was slower than in proantithrombin. This again suggests the presence in whole serum of a substance inhibitory for the growth of an avirulent strain.

The results with the vitamins do not, in general, indicate any marked differences between the virulent H37Rv and the avirulent H37Ra. It is apparent that the observations reported in the present study cannot explain the difference in response to serum on the basis of a difference in vitamin requirements. The inhibitory effect of high concentrations of the vitamins noted in this study confirms the findings of Koser and Kasai (23, 24). They have shown that a number of organisms, including pneumococci, various strains of *Streptococci* and of the enteric bacilli, were markedly or completely inhibited by concentrations of 5,000 or 10,000 γ of nicotinic acid or nicotinamide per ml. Niacinamide has, in fact, been found to have tuberculostatic activity for H37Rv both *in vivo* and *in vitro* (25, 26).

In the experiments described herein, the effect on the growth rate of the particular substrates employed indicates that differences in metabolism exist between the strains tested. There are indications that the virulent strain is able to utilize substrates present in whole serum to a greater advantage, in the sense that the growth rate is stimulated to a greater degree, than the avirulent variant derived from the virulent strain. Further study will be necessary to establish what relationship, if any, these findings have with the property of virulence. The findings, if verified, that virulent strains possess enzymatic systems which better enable them to adapt to nutrients in the body of the host than their

avirulent counterparts would be of great significance in explaining in part the difference in ability to grow and multiply *in vivo*.

SUMMARY

The minimal inoculum technique has been applied to a study of the effect of serum, plasma fractions, and a number of vitamins on the growth of virulent and avirulent strains of tubercle bacilli. By this method, the growth rate of H37Rv was compared with that of its avirulent variant, H37Ra, by determining the effect of substrates in various concentrations when added to a chemically defined Proskauer and Beck medium.

The two strains were found to have the same generation time in chemically defined medium. The growth rate of H37Rv was stimulated by the addition of serum, whereas the growth rate of H37Ra was the same in serum as in the Proskauer and Beck medium.

Because of the results obtained in serum medium, twelve plasma fractions were tested for their effect on the growth rate of H37Ra and compared with those obtained for H37Rv. Of these, four fractions were found to stimulate the growth of H37Rv enough to account for the increased growth rate in serum medium. These were proantithrombin, fraction V albumin, crystalline albumin, and antifibrinolysin. Two of these fractions, proantithrombin and fraction V albumin, decreased only to a slight extent the generation time of H37Ra over that in serum medium or in chemically defined medium.

Neither H37Rv nor H37Ra produced in a chemically defined medium within twenty-one days a substance which inhibited or stimulated the growth of either strain in the concentration employed. In the filtrate of both strains grown in proantithrombin, a significantly decreased generation time was found for strain H37Ra. The effect on the growth rate of H37Ra, however, was not as great as on the growth rate of H37Rv.

No marked effect on the growth rate of either H37Rv or H37Ra was found in the presence of any of ten vitamins or of coenzyme I which could explain the difference in response to serum.

In general, the avirulent variants of human and bovine mammalian strains were found to be more metabolically deficient, and none showed the same response to serum and the plasma fractions as the virulent H37Rv strain.

In the experiments described, the effect on the growth rate of the particular substrates employed indicates that differences in metabolism exist between the strains tested.

SUMARIO

Estudios del Metabolismo de las Micobacterias Virulentas y Avirulentas

La técnica del inóculo mínimo fué aplicada a un estudio del efecto del suero, de fracciones del plasma y de varias vitaminas sobre la proliferación de cepas virulentas y avirulentas de bacilos tuberculosos. Con este método, se comparó la velocidad del desarrollo de H37Rv con la de la variante avirulenta de la misma, H37Ra, determinando el efecto de subestratos agregados a varias concentraciones a un medio químicamente bien definido de Proskauer y Beck.

Las dos cepas mostraron el mismo tiempo de generación en el medio químicamente bien definido. La velocidad del desarrollo de H37Rv fué estimulada por la adición de suero, en tanto que la de H37Ra fué en el suero la misma que en el medio de Proskauer y Beck.

Debido a los resultados obtenidos en el médio sérico, se comprobaron doce fracciones de plasma en cuanto a su efecto sobre el índice de desarrollo de H37Ra, comparando el resultado con el obtenido con H37Rv. De ellas, observóse que cuatro fracciones estimulaban el desarrollo de H37Rv lo suficiente para explicar el índice mayor observado en el medio de suero, siendo las mismas proantitrombina, albúmina de la fracción V, albúmina cristalina y antifibrinolisina. Dos de esas fracciones, la proantitrombina y la albúmina de fracción V, apenas hicieron descender el tiempo de generación de la H37Ra, comparado con el observado en el medio sérico o en el químicamente bien definido.

Ni la H37Rv ni la H37Ra produjeron en un medio químicamente bien definido en término de veintiún días una substancia que inhibiera o excitara el desarrollo de una u otra cepa a la concentración empleada. En los filtrados de ambas cepas cultivadas en proantitrombina, notóse un tiempo de generación significativamente menor para la cepa H37Ra. Sin embargo, el efecto sobre el índice de desarrollo de H37Ra no fué tan grande como sobre el de H37Rv.

No se notó ningún efecto pronunciado sobre el desarrollo bien de H37Rv o de H37Ra en presencia de ninguna de diez vitaminas o del co-encima I que pudiera explicar la diferencia en la respuesta al suero.

En general, las variantes avirulentas de las cepas tanto humanas cuanto bovinas de mamíferos resultaron ser más deficientes metabólicamente, sin que ninguna mostrara la misma respuesta que la cepa virulenta H37Rv al suero y a las fracciones de plasma.

En los experimentos descritos, el efecto sobre el índice de desarrollo de los subestratos utilizados indica que existen diferencias del metabolismo entre las cepas ensayadas.

RESUME

Etude du métabolisme de mycobactéries virulentes et avirulentes

La méthode de l'inoculum minimal a été appliquée à l'étude de l'action du sérum, de fractions du plasma et d'un certain nombre de vitamines sur le développement de souches virulentes et avirulentes de bacilles tuberculeux. Par cette méthode, le taux de croissance de la souche H37Rv a été comparé avec celui de sa variante avirulente, H37Ra, par la détermination de l'action de substrats, en concentrations différentes, ajoutés à un milieu de Proskauer et Beck chimiquement défini.

Le temps de reproduction a été trouvé le même pour ces deux souches cultivées dans un milieu chimiquement défini. Le taux de croissance de H37Rv a été augmenté par l'adjonction de sérum, tandis que le taux de croissance de H37Ra a été le même dans le sérum et dans le milieu de Proskauer et Beck.

En raison des résultats obtenus dans le milieu sérum, douze fractions de plasma ont été soumises a des tests destinés à déterminer leur action sur le taux de croissance de H37Ra, par comparaison avec les résultats obtenus avec le H37Rv. Quatre de ces fractions ont provoqué une stimulation de la croissance de H37Rv, qui suffit à expliquer l'accroissement du taux de croissance dans le milieu sérum. Ces fractions étaient: la proantithrombine, la fraction albumine V, l'albumine cristallisée, l'antifibrolysine. Deux de ces fractions, la proantithrombine et la fraction albumine V ne réduisaient qu'à un faible degré le temps de reproduction de H37Ra, par rapport à celui obtenu avec le milieu sérum ou le milieu chimiquement défini.

Aux concentrations utilisées, la souche H37Rv de même que la H37Ra, cultivées dans un milieu chimiquement défini, n'ont jamais produit, dans les vingt-et-un jours, une substance inhibant ou stimulant la croissance de l'une ou l'autre de ces souches. Dans le filtrat de ces deux souches cultivées dans la proantithrombine, on a observé que, pour la souche H37Ra, le temps de reproduction était abaissé de façon significative. L'action exercée

sur le taux de croissance de H37Ra était toutefois moins grande que sur le taux de croissance de H37Rv.

En présence de l'une quelconque des dix vitamines ou coenzyme I il n'a été observé aucune action marquée sur la croissance de H37Rv ou de H37Ra qui permette d'expliquer la différence dans la réaction au sérum.

D'une manière générale, les variantes avirulentes des souches humaines et bovines ont été trouvées plus déficientes, du point de vue métabolique; aucune d'entre elles n'a montré la même réaction au sérum et aux fractions de plasma que celle de la souche virulente H37Rv.

Dans l'expérimentation décrite, l'action sur le taux de croissance des substrats particuliers, qui ont été utilisés, montre qu'il existe des différences dans le métabolisme des souches étudiées.

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THE PREVENTION OF DEFORMITIES FOLLOWING THORACOPLASTY[1,2]

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(Received for publication May 12, 1952)

INTRODUCTION

Post-thoracoplasty deformity has often been considered to be an unavoidable complication of the operation. Two factors have been responsible for the continuation of this fatalistic attitude: first, surgeons, being primarily interested in control of the disease, were slow to realize the necessity for postural correction; second, physical medicine is a comparatively new science and trained workers have not been available to carry on a widespread exercise program.

Linduff (1) recognized that the experience gained in nontuberculous thoracic fields during World War II could also be applied to the surgery of tuberculosis. Her description of the typical postoperative deformity is commonly accepted. This consists of lateral deviation of the head and neck to the nonoperated side; elevation and anterior rotation of the shoulder on the operated side; elevation of the pelvis on the nonoperated side; scoliosis, with the thoracic convexity on the operated side and a compensatory lumbar curve in the opposite direction. She stated also:

> Clinical experience has shown that when selected exercises are properly done they do not interfere with collapse therapy, do not increase the excursion of the infected lung, or result in untoward reactions.

The necessity for initiation of a coordinated program was emphasized by Triester (2), who stated that deformity begins to develop immediately following the first stage and that corrective measures must therefore be instituted early. Restoration of the scapulohumeral joint function has been the main objective of many programs. Attempts have been made by others to maintain the shoulders and hips level while both are in vertical alignment.

It is recognized that the objective of a thoracoplasty is to aid in the arrest of the disease. The deformity which too often has accompanied it, however, is not necessary. To some patients it has acted as a bar to complete social adjustment. Others have been dissuaded from accepting the operation by the fear of such a deformity.

Since the responsibility of the practitioners, as a team, is to the whole patient rather than to an apical cavity, it is essential to devise a program which will discharge the total obligation to the patient. The ideals of such a program can be attained only when a well-adjusted patient is returned to society with a

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[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

minimal postoperative deformity. Fear of causing an exacerbation of the underlying pulmonary lesion has frequently led to avoidance of the strenuous type of resistive exercises which have been found necessary to prevent or minimize this deformity.

The early program in this clinic was usually successful in promoting good scapulohumeral function. Considerable attention was devoted also toward efforts to derotate the spine because of the commonly held belief that scoliosis was due to rotation of the vertebral bodies. In spite of these efforts, deformities resulting from scoliosis and pelvic tilt were common sights on the wards. In 1948, a resistive exercise program was initiated. This has been developed, step by step, until at the present time it is possible to attack all components of the deformity with considerable success. The steps in the evolution of that program are covered in succeeding paragraphs.

OBSERVATIONS

Steps in Development of Program

It was noticed, early in the study, that some patients were left with a maximum and others with a minimum postoperative deformity. In an effort to determine whether factors such as age, sex, race, body type, or extent of operation would furnish a reason for the disparity in degree of deformity, a photographic review of 70 patients was made. From this review it was apparent that older individuals with fibrotic ligamentous tissues and younger ones with good muscular development had the least postoperative scoliosis.

Another study of a group of 12 patients who were to have a thoracoplasty was then begun. Photographs were taken of these patients before the initial operation, before each subsequent stage, and routinely as significant changes occurred. The exercise regimen prescribed for these patients included:

Straight alignment of the body in bed.
Full range of motion to the operated shoulder three times daily.
Manual resistance exercises for prevention of scoliosis by strengthening all muscle groups on the operated side three times daily.
Pulse and respiratory rates were recorded before exercise, immediately after, and five minutes afterwards. The interesting observation was made that both rates were lower five minutes after, than they were prior to, exercise.

The patients in this group cooperated well in carrying out the prescribed regimen. Improved function of the shoulder girdle was easily demonstrated. Overdevelopment of the lower trunk muscles on the operative side was also obtained, but the scoliosis was not markedly decreased.

An effort was made to determine how much muscle pull was required to derotate the spine by examination of a body with a typical post-thoracoplasty deformity. In this examination the therapist was unable to demonstrate that rotation of the vertebral bodies was a contributory cause of the postoperative scoliosis. It was observed that the *Psoas major* muscle on the operated side was larger than on the nonoperated side. The most important result of the study

was the discovery, that, when the shoulders were lifted and shifted toward the unoperated side, the dorsal and lumbar spine straightened. The therapist then remembered that 2 of the patients who, contrary to instructions, had persisted in lying in this lifted and shifted position had less scoliosis than many patients who had cooperated completely. The position of the 12 patients in that study was then changed to what will from now on be referred to as the "overcorrected" position.

In searching for an explanation for the apparently unorthodox effect of the "overcorrected" position on the spine, it was noted that there are no long muscles which have both their origin and insertion on thoracic vertebrae one through eight. There are, however, muscles originating in the cervical vertebrae which insert on these thoracic vertebrae. These muscles (*Erector spinae*, *Transversalis colli*, *Semispinalis colli*) on the unoperated side were used for traction on the unsupported vertebrae in the operative area when the head was laterally flexed toward the operative side. Likewise, there are muscles which originate from the lower dorsal and lumbar regions and insert into the first eight thoracic vertebrae, such as the *Erector spinae* group. These muscles on the unoperative side applied traction to the unsupported vertebrae in the operative area when enough lateral thoracic shift was obtained by the position of the trunk. It was also found that forward flexion of the head applied tension to the vertebrae in the operative area through the interspinous and supraspinous ligaments. The forward and lateral flexion of the head to the operated side, combined with the lateral shift of the thorax to the unoperated side, was later termed "passive overcorrection" to differentiate it from "active overcorrection" described in later phases of the program. Shortly after the bed positioning was changed to "overcorrection," it was observed that the exercises administered to develop the transsected rhomboid and trapezius muscles actually produced the greatest development in the contralateral lower spinalis group. As the contralateral spinalis contracted to stabilize the spine so that the upper extremity on the operated side could function, the spine appeared to overcorrect in much the same manner as with the "passive overcorrection" positioning. An attempt was then made to find other movements which would strengthen the lower spinalis on the unoperated side. Two or three exercises were tried but discarded because it was difficult to surrender *the belief that the muscles on the side of the concavity are strong and produce the curve.*

Even though manual resistance exercises were being applied to the transected muscles, little result could be observed other than that the inferior angle of the scapula appeared to approach the last remaining rib as its superior border rolled or tilted forward and downward, thus changing the contour of the shoulder to a more nearly normal one. So marked was this change that the therapist remarked to a patient that it would be desirable to determine which muscles produced the change. If these muscles were known, exercises could be directed toward their development. This, in turn, would aid in maintaining the optimal position of the scapula. The patient said that, if he performed the exercise in front of a mirror, the contracting muscles might be seen. When this was done,

the tensed *Pectoralis minor* muscle became clearly visible. Several exercises were then devised, directed toward the overdevelopment of all digitation of the *Pectoralis minor*. In order to further free the scapula so that the inferior angle could rest outside the remaining rib, massage of the adherent scar tissue was administered as soon as permitted by the surgeon.

The conclusions reached in the preliminary study caused the present investigators to direct their efforts toward the development of an exercise program for the trunk muscles of the unoperative side. In combining the "overcorrected" bed position with this routine of exercises, better results were obtained in the 12 patients composing the preliminary study group than had been obtained in any other group which had been treated.

As a consequence of these improved results, an expansion of the roentgenographic studies was undertaken. Films of the cervical, thoracic, and lumbar spine were obtained before surgery and before each stage as a routine measure. These sectional films were joined so that the entire spine could be viewed as in one film. It was then seen that the curve which caused the deformity took the shape of an elongated "C" (figure 1), beginning at the first cervical vertebra and ending near the third lumbar vertebra. The greatest convexity was at the level of the third, fourth, or fifth dorsal vertebra, depending upon the number of ribs removed. In some patients a slight lower lumbar compensatory curve was present which did not contribute to the deformity.

Recognition of the elongated "C" curve stimulated further review of anatomy and muscle testing and resulted in the discovery that the *Quadratus lumborum* was weaker on the nonoperated side. All patients were then instructed in an exercise designed to produce maximal contraction and development of this muscle. A roentgenographic checkup after two months showed that marked improvement in vertebral alignment had resulted. A similar analysis was made of the muscles at the upper end of the "C" curve, and finally the lateral neck flexors of the nonoperated side were brought into the concept. After two years of trial and error, the program of development of the contralateral trunk and neck muscles was complete. The procedures thus developed seemed very simple and logical in retrospect. The entire problem was now a familiar one of "passive overcorrection" or "splinting" and "active overcorrection" or exercise to maintain this position with normal tension.

Influence of Exercises on Pulmonary Lesions

As the program developed, it became apparent that the proponents would face a barrage of criticism unless it could be proved to the physicians concerned with the treatment of pulmonary tuberculosis that the exercises were not harmful. A study was made, therefore, of a group of 72 consecutive patients who had had thoracoplasties in 1946 (before the initiation of the resistive exercise program) and of 55 patients who were operated upon after adoption of the exercise program in 1948.

An evaluation was made one year after the final stage of surgery in both groups. It was found that, in the 1946 nonexercise group, 55.4 per cent had

sputum negative for *M. tuberculosis* one year after operation; 71 per cent of the 1948 exercise group, on the other hand, had sputum negative for tubercle bacilli. Complications such as postoperative spreads of disease, atelectasis, and wound infection, as well as the case-mortality rate, were higher in the nonexercise than in the exercise group.

FIG. 1. Typical "C" curve following a seven-rib left thoracoplasty

In the 1948 study, an evaluation of the immediate effects of exercise was also made. The temperature and the pulse and respiratory rate of each patient were charted before, immediately after, five minutes after, and one hour after exercise. The temperature remained within normal limits at all times. Pulse and respiratory rates were slightly increased immediately after exercise but returned to their original rate within five minutes in every patient.

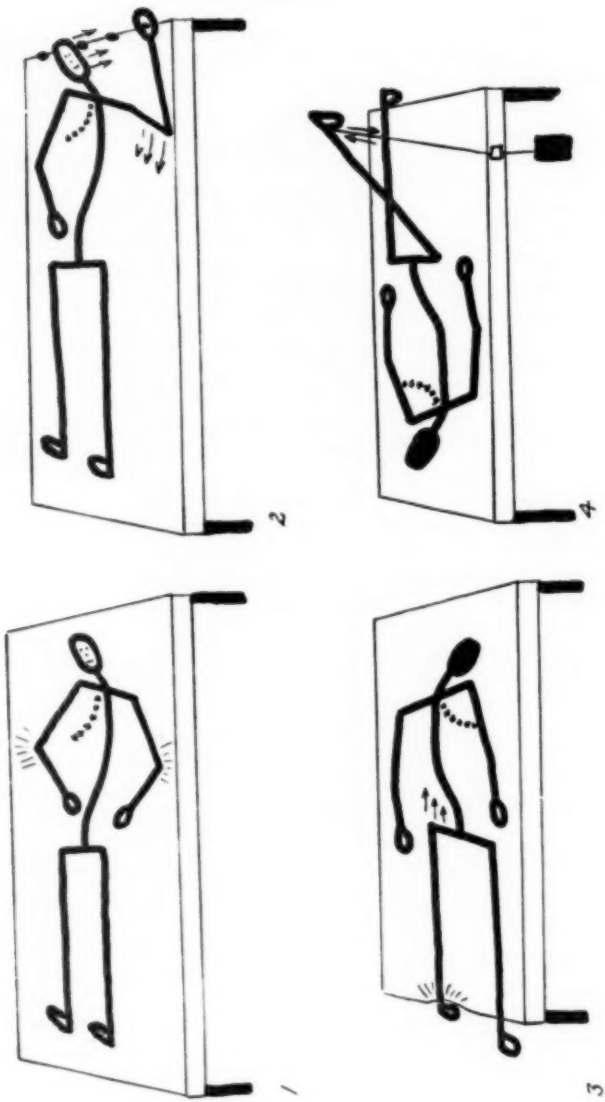
DISCUSSION

It is not intended to convey the impression that the exercise program is a treatment for pulmonary tuberculosis. Improvements in anesthesia, surgical techniques, and postoperative care are undoubtedly responsible for the improvement in end results. The present writers were convinced, however, that resistive exercises could be used in the early postoperative period without harmful effects upon the pulmonary lesion. By the use of this exercise program, skeletal deformities were minimized or eliminated in a majority of the thoracoplasty patients operated upon after the adoption of the 1948 exercise program. The mental outlook of the latter group of patients was improved. The fact that a definite program had been designed to carry them through their entire operative period served as a morale builder and helped the staff to keep the majority of these patients in the hospital for ten to twelve months postoperatively. In former years the percentage of surgical patients who remained to obtain an arrested status was relatively low.

In the present study, anatomic causes for post-thoracoplasty deformity had been analyzed and the fundamentals of the corrective measures defined. The postural changes which occurred immediately following surgery, were: (*1*) contralateral deviation of the head and neck, (*2*) ipsilateral convexity of the spine in the operative area, the convexity extending down to the lower dorsal and upper lumbar area in the form of an elongated "C" curve. Further postural changes occurred following the removal of ribs. The scapula lost its anterior skeletal support and the function of its scapulothoracic joint. Its inferior angle rotated toward the coronal plane and often became fixed under the first remaining rib. This fixation was maintained by internal fibrinous membranes and adherent external scar tissue. The acromioclavicular joint on the operated side was elevated and rotated anteriorly.

It was now apparent that the deformity problem was in reality two problems. The primary cause of deformity was the transection of muscle groups and removal of ribs in the operative area. This caused a disturbance in balance of the vertebral column which resulted in scoliosis. The second and minor portion of the deformity problem was the scapulohumeral. It is the current belief of the present writers that the spinal imbalance must be attacked first. Resistive exercises are used to strengthen and re-educate the contralateral muscle groups as the vertebral column is straightened and tensed by active and passive overcorrection. The muscles which are chiefly concerned in the spinal phase of the deformity problem are the trapezius, the rhomboids, the *Latissimus dorsi*, the *Erector spinae*, the *Transversalis colli*, the *Semispinalis colli*, and the *Quadratus lumborum*.

The spinal deformity begins immediately, even on the operating table. Therefore, corrective measures must be initiated on the first postoperative day. The unorthodox approach in the present study has been to direct maximum efforts toward development and education of the muscles of the contralateral side. When these muscles are properly conditioned and the spine straightened and tensed, they will of necessity exert their greatest efforts on the central portion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of the spinal concavity in the operative area which coincides with the midpoint of the elongated "C" curve. The program of exercises must be continued throughout the entire period of hospitalization. The patient must be so indoctrinated that the "overcorrected" position will become a part of his daily life in the early post-hospital years.

The incarceration of the lower angle of the scapula under the first rib and the elevation of the operated shoulder are more apparent to the patient than is the scoliosis. They give him more concern in the early postoperative days than does the vertebral problem. In the opinion of the writers, however, they are of secondary importance.

When the scoliosis has been corrected and the spine stabilized, efforts are intensified to solve the scapulohumeral problem. Training of the patient in the development and use of the *Pectoralis minor* muscle is of the utmost importance. When this muscle has been properly conditioned and the internal and external scar tissue in operated area softened by massage, he is able to obtain free motion of the scapula and to roll the inferior angle out from its anterior placement. While the early exercise program is designed to bring the *Pectoralis minor* into play, serious efforts to release the incarcerated scapula are not made until approximately eight weeks after operation. The orthodox exercises to obtain complete range of motion of the shoulder joint are carried out from the first postoperative day.

The program to be described in the following pages cannot be minute in detail. It will supply an outline of the plan and, by means of diagrammatic drawings, will furnish the technique of selected exercises used during each postoperative phase. The success of the program depends upon teamwork, and the team concept must be conveyed to all physicians, nurses, therapists, and hospital aides who come in contact with the patient. It will be discouraging to a patient if his ward physicians and nurses are not familiar enough with the problem to encourage and guide him through the trying days of his convalescence.

For this reason, an orientation course of lectures is given periodically to all hospital personnel concerned with patient care. Roentgenograms are shown,

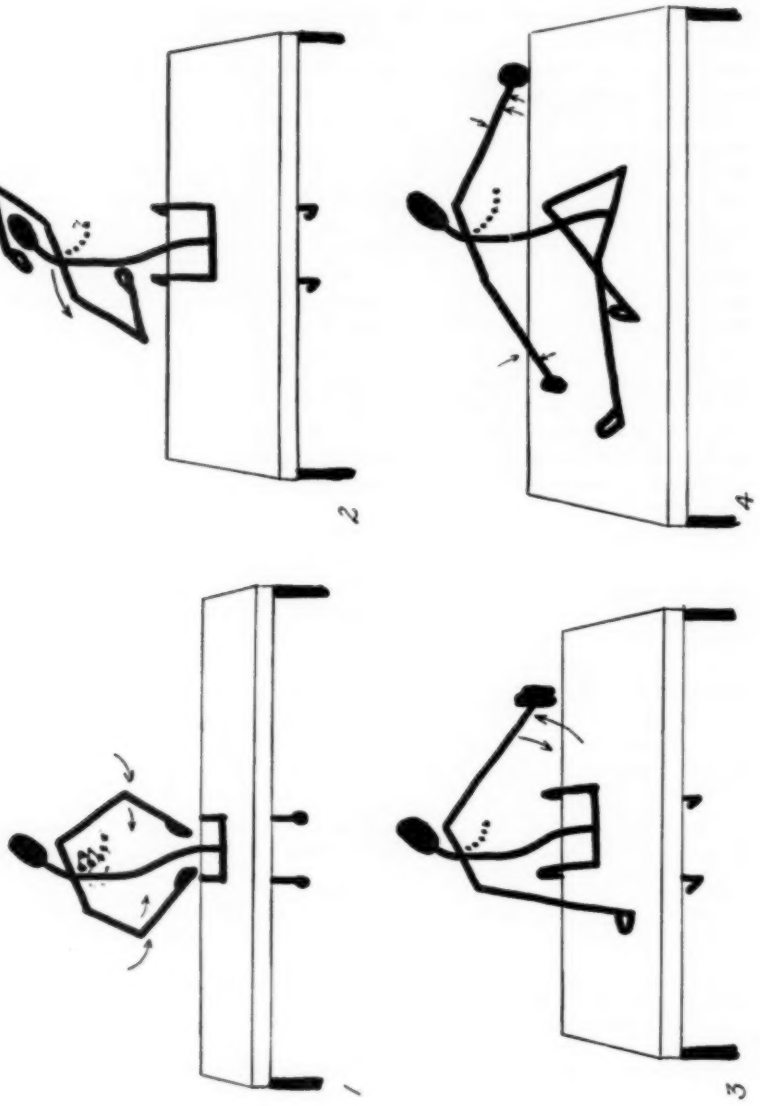
FIG. 2. Recumbent

1. Lying supine in overcorrected position with hands on hips, push both elbows into the mattress to the limit of strength and pain to overdevelop *Pectoralis minor* on operated side and strengthen contralateral back muscles.

2. Lying supine in overcorrected position, with head between posts at head of bed, abduct arm of unoperated side slightly above shoulder level and grasp bedpost with hand of unoperated side. Pull flexed elbow of unoperated arm toward the hip and push head against bedpost toward unoperated side to maximum strength. By using the head and unoperated hand as points of stabilization, the *Erector spinae* group and *Latissimus dorsi* contract to their maximum strength to shift the spine toward the unoperated side.

3. Lying prone in overcorrected position, with feet over the end of the mattress, pull hip of unoperated side toward the ribs. The mattress supplies resistance when foot on unoperated side is pulled against it for maximum contraction of *Quadratus lumborum*.

4. Lying prone in overcorrected position, with pulley attached to ankle of unoperated side, hyperextend thigh, with knee straight, against resistance of pulley to maximum height to obtain maximum contraction of *Erector spinae* muscles on unoperated side.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the deformity explained, and the reasons for the steps taken for correction made clear. A motion picture film in color, with narration in sound, has been prepared which presents the work in a logical and continuous manner. The showing of this film has simplified the personnel orientation.

Preoperative briefing of the patients is one of the most important steps in the development of the program. An informed patient is usually a cooperative one. If he knows that a definite plan has been devised to follow him through his entire postoperative hospital course, he will usually be an enthusiastic patient.

The preoperative orientation course consists of: (*1*) showing the moving picture described above, (*2*) teaching bed positions by means of a mirror (designed by the therapist at this hospital), (*3*) teaching the exercises which will follow immediately after surgery. At this briefing the patient is given an opportunity to ask any questions which might pertain to the program. Above all, he is left with the thought that the problem is largely his own and that the staff can only guide him in the steps that will be necessary to correct or minimize the postoperative deformity. He is told that it is not a problem of a week or a month but one that must be actively a part of his life for several years after discharge.

The exercise program is divided into three stages: the recumbent, the semi-weight-bearing, and the weight-bearing. The patient is instructed that each exercise is to be repeated only five times at three different periods of the day. All exercises are to be performed in the "overcorrected" position. Weights sufficient to produce a maximum contraction of the muscle are used for each exercise. These weights may vary from the force of gravity to 15 pounds in the recumbent stage. In the semi-weight-bearing stage, a maximum of 20 pounds is used. At the end of the weight-bearing stage, as the time for discharge from the hospital is approached, 50 pounds may be used.

Exercises for the recumbent stage are used in the postoperative period while the patient is confined to bed. They begin on the first postoperative day and are carried through until he reaches the second stage, or semi-weight-bearing. Some of the exercises of the

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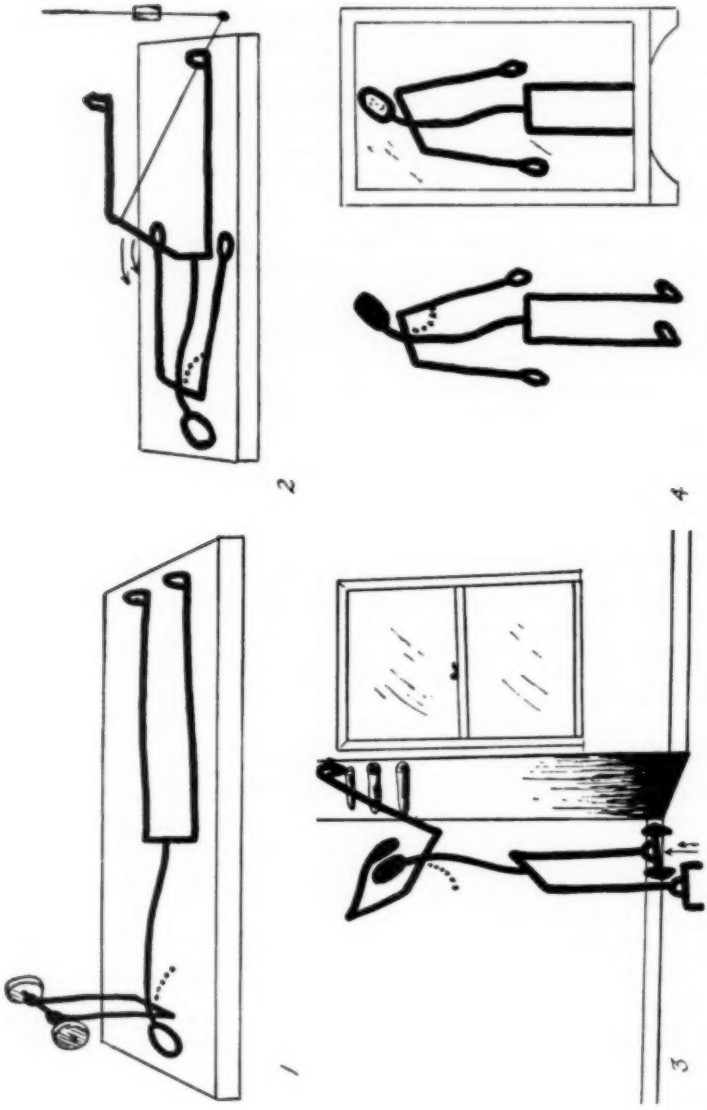
FIG. 3. Semi-weight-bearing

1. Sitting overcorrected with hands on hips, push both elbows back against manual resistance to limit of strength to strengthen *Pectoralis minor* on operated side and contralateral back muscles.

2. Sitting overcorrected with hand of operated side extended overhead with palm over ear of unoperated side, hand of unoperated side against lower lateral chest of same side. With therapist offering resistance at base of neck of unoperated side, patient attempts to laterally flex cervicodorsal spine and produce maximum contraction of upper and lower contralateral trunk muscles.

3. To gain maximum contraction of muscles in operated shoulder girdle and contralateral back muscles, patient sits overcorrected with weight of body supported posteriorly on arm of unoperated side, abducts arm of operated side to shoulder level only, against maximum resistance of weights.

4. Sitting in overcorrected position with the weight of the body on the hip of the operated side and leg of operated side flexed under that of the unoperated side, extend leg of unoperated side sideward in line with the trunk, lift arms, with maximum amount of weight, to shoulder level and bend body to unoperated side for maximum contraction of the contralateral trunk muscles.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recumbent stage are shown in diagrammatic form by means of the accompanying figures, which are self explanatory (figure 2).

In the noncomplicated case, bathroom privileges are given at the end of the third month postoperatively. At that time he is given the exercises designed for the semi-weight-bearing stage. Some of the exercises used in the semi-weight-bearing stage are illustrated in figure 3.

At the beginning of the fifth month, the patients are allowed to be out of bed for three hours daily. The patient is then permitted to go to the central physical medicine clinic for the exercises of the weight-bearing stage. Some of the activities depicted in the accompanying diagrams (figure 4) are carried out in the clinic for a period of two months, following which the patients progress to the rehabilitation center. There, for a period of four to six months, the patient advances in postural re-education through the various shop courses, continuing in the meantime the exercises of the weight-bearing stage.

Occupational therapists participate in the program and have devised activities which further enhance the principles outlined in the present report. One example of this is the use of the weighted loom. This provides exercises for all of the muscle groups on the nonoperated side while engaged in productive labor. Thus, the patient establishes "overcorrected" habit patterns which he will carry on in his daily activities after he leaves the hospital. Not only have these new methods in occupational therapy helped to minimize deformities, but they have contributed to the build-up of the patients' morale.

SUMMARY

It has been found that correction of the post-thoracoplasty deformity presents two problems. Disturbance in spinal balance results in scoliosis, which is treated by means of active and passive overcorrection. A program of resistive exercises for the contralateral trunk muscles is carried out in the "overcorrected" position. The scapulohumeral phase of the problem is of secondary importance. It is treated by range of motion exercises, resistive exercises for the *Pectoralis minor* muscle, and massage of the scar tissue. The team concept is of the utmost importance in maintenance of patient morale. Since the adoption of this regimen it has been possible to prevent or minimize postoperative deformity without harmful effect upon the pulmonary lesion.

FIG. 4. Weight-bearing

1. Lying supine in overcorrected position, push weighted chest bar upwards, fully extending the arms, for maximum contraction of pectoral muscles.

2. Lying supine in overcorrected position, with feet toward weighted pulley, attach pulley above knee of unoperated side and pull knee towards chest against weight for maximum contraction of *Psoas major*.

3. Standing on footstool in overcorrected position with unoperated side towards stall bars, extend hand of operated side overhead and, with palm over ear of unoperated side, grasp bar with hand of unoperated side at level of maximum reach; pull hip on unoperated side upward against the resistance of foot weighted by iron boot to gain maximum contraction of *Quadratus lumborum*.

4. Practice sitting and standing overcorrected in front of mirrors at least five minutes per day.

SUMARIO

La Prevención de Deformidades Consecutivas a la Toracoplastia

La rectificación de toda deformación posttoracoplástica plantea dos problemas. El trastorno del equilibrio raquídeo acarrea escoliosis, que se trata por medio de la hipercorrección activa y pasiva, llevándose a cabo un plan de ejercicios resistivos para los músculos contralaterales del tronco en la posición "hipercorregida." La fase escápulo-humeral del problema reviste importancia secundaria, y es tratada por medio de ejercicios de acción, ejercicios resistivos para el pectoral menor y masaje del tejido cicatricial. En el mantenimiento del estado de ánimo del enfermo, el concepto de cooperación y colaboración reviste importancia suprema. Desde la implantación de este régimen, ha sido posible impedir o reducir al mínimo la deformidad postoperatoria sin afectar desfavorablemente la lesión pulmonar.

RESUME

Prévention des déformations succédant à la thoracoplastie

Les auteurs ont observé que la correction des déformations qui se produisent à la suite d'une thoracoplastie présentaient deux problèmes. Le déséquilibre du rachis provoque une scoliose, qui est traitée par une hypercorrection active et passive. Un programme d'exercices de résistance à la contraction des muscles contro-latéraux du tronc est mis en pratique, en position "hypercorrigée." La phase scapulo-humérale du problème est d'importance secondaire. Celle-ci est traitée par une série d'exercices gradués, exercices de résistance pour le petit pectoral et massage des tissus cicatriciels. Le concept "équipe" est d'une importance considérable pour le maintien d'un bon moral chez le malade. Depuis l'adoption de ce programme, il a été possible de réduire au minimum la déformation post-opératoire sans production d'effets nocifs sur la lésion pulmonaire.

Note

The concept of the exercise program devoted chiefly to the contralateral muscle group was worked out by Ila J. Amrhein, Chief Physical Therapist.

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A STUDY OF PAIN THRESHOLD IN TUBERCULOUS PATIENTS[1, 2]

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(Received for publication April 21, 1952)

INTRODUCTION

Much has been written concerning the interrelationship of tuberculosis and various mental and emotional phenomena. Berle reviewed the literature pertaining to this subject and emphasized that most studies have been confined to clinical evaluation, inadequately controlled, and "impressionistic" (1). She recommended that future investigations be conducted in such a way as to permit the use of methods based on the recording of "measurable physiologic changes" associated with emotional stress, as has been done in other disease states.

Using the Hardy-Wolff-Goodell pain threshold apparatus, Chapman and coworkers (3, 4, 5) demonstrated that psychoneurotic patients had a significantly lower mean pain reaction threshold than control subjects who presumably were non-neurotic. Schilling and Musser (18, 19, 20) confirmed this observation and also reported that, in one series of patients with peptic ulcer and in another with "menopausal syndrome," the mean pain reaction threshold closely corresponded to that of a psychoneurotic group. They pointed out that "failure to show a spread between perception and reaction threshold is strong evidence for the existence of a significant emotional disturbance."

The implication from these studies is that the subject under emotional stress may be hyperreactive to noxious stimuli, whereas those relatively free from such stress are not.

The present investigation was undertaken to compare tuberculous and general medical patients in terms of their pain thresholds and to correlate the results in the tuberculous subjects with such factors as mental status and extent and duration of the pulmonary disease.

METHODS AND MATERIALS

The Hardy-Wolff-Goodell apparatus has previously been described in detail (11, 18). Essentially, this is a device for delivering a controlled, measured amount of radiant energy to a properly prepared skin surface so that the level of pain perception and pain reaction can be determined. By using a pair of lenses, the light from a rheostatically controlled 500-watt incandescent lamp is focused through a fixed aperture onto the skin-test site. An electrically operated shutter provides for a standard exposure time of three seconds. An absorption coefficient

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[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

of unity is ensured by blackening the skin with india ink sufficiently long before testing to allow for complete drying.

A voltmeter is attached across the lamp circuit. The voltage readings are recorded when the subject first perceives pain and again when he reacts to it. Since the energy output for any given voltage is known, the voltmeter readings are later translated into energy units, gram calories per second per square centimeter.

In this investigation all subjects were given similar instructions prior to testing. They were informed what the testing device was and how it operated. They were told that sometime during the series of trials they would feel a sharp, stinging, pricking, or jabbing sensation (sometimes described as a "hot needle") and that as soon as they perceived this they were to comment on it. It was pointed out that the sensation of warmth was *not* the desired end point. Reaction to the stimulus by wincing or withdrawing was not mentioned before the experiment. Ordinarily, a complete experiment on one person entailed ten to fifteen exposures of varying intensity of stimulation using the mid-forehead and dorsum of the hand alternately. All of the tests were performed by the same operator and, as nearly as possible, under basal conditions. None of the patients received analgesics on the day of testing.

The pain *perception* threshold was taken as the lowest level of stimulation at which the subject experienced a sharp, stinging, or pricking sensation; whereas the pain *reaction* threshold was considered as the lowest level at which a motor response was noted, i.e., a wince or a withdrawal.

The subjects were obtained from two sources: the Veterans Administration Hospital and the University Hospitals, Madison, Wisconsin.

In an effort to avoid the matter of selection, all 30 patients on one (male) ward of the VA Hospital served as the tuberculous group. Each of these patients had been proved by various clinical and laboratory means to have active pulmonary tuberculosis. The secondary diagnoses, however, were unknown to the investigators until after completion of the experiment. The age range of these patients was from twenty to fifty-seven years.

The 30 general medical patients were occupants of a single, male medical ward at the University Hospitals. Here some selection was carried out for the purpose of obtaining a comparable group. Patients less than twenty and more than sixty years of age were avoided. Those with a *primary* diagnosis of psychoneurosis or peptic ulcer were excluded. This was deemed necessary since the tuberculous patients, regardless of antecedent or coincident emotional disorders, were hospitalized because they had tuberculosis; not for psychiatric reasons. Thus, the general medical patients selected for study were those who were hospitalized with a primary diagnosis of some "organic" disease including: carcinoma, leukemia, anemia, cirrhosis, diabetes, rheumatoid arthritis, hypertensive cardiovascular disease, et cetera. It was not assumed that this manner of selection eliminated from the series those with emotional disorders; indeed, the presence of psychiatric and emotional disturbances in some of these patients was anticipated. However, under the prescribed circumstances, any existing emotional factors were regarded as bearing a relationship to the over-all medical problem not unlike that in the tuberculous group.

Some time after completion of experimental observation the clinical record of each patient was reviewed and a note made regarding secondary diagnoses, particularly those of psychoneurosis or other neuropsychiatric disorder. In cases where there was question, the patient was interviewed by a trained observer so that a positive diagnosis could be established. Additionally, in the tuberculous patients, the extent and the duration of their pulmonary disease were recorded.

RESULTS[2]

Comparison of tuberculous and general medical patients: As illustrated in table 1, the mean pain *perception* threshold for the tuberculous patients was 0.303 gm. calories per second per square centimeter and the mean for the general medical patients was 0.292 gm. calories per second per square centimeter. The difference

TABLE 1

PAIN THRESHOLDS OF TUBERCULOUS AND GENERAL MEDICAL PATIENTS

GROUP	TUBERCULOUS	GENERAL MEDICAL
Number	30	30
Age (average)	38 years	45 years
Neuropsychiatric diagnosis	13	8
Pain perception threshold (mean) in gm. cal./ sec./ cm.^{2}	0.303	0.292
Pain reaction threshold (mean) in gm. cal./sec./ cm.^{2}	0.433	0.430

The difference in the mean perception and reaction thresholds of the two groups is not statistically significant.

in these means is not significant. Likewise, there was not a significant difference between the mean pain *reaction* thresholds in these two groups; the values being 0.433 gm. calories per second per square centimeter for the tuberculous subjects and 0.430 gm. calories per second per square centimeter for the general medical patients.

Comparison of tuberculous patients with and without neuropsychiatric diagnoses: Among the 30 patients with pulmonary tuberculosis, 13 had neuropsychiatric diagnoses. Six were classified as having character disorders, the major manifestation of which was chronic alcoholism, 2 had mixed psychoneurotic reactions, 4 were diagnosed as having anxiety reaction, and one patient had multiple sclerosis (table 2).

When the pain thresholds of the 13 patients with neuropsychiatric (NP) diagnoses were compared with those of the remaining 17 tuberculous subjects, it was found that their mean perception levels were similar; however, the mean pain *reaction* threshold of the NP group was significantly lower than that of the non-NP group. Likewise, the mean difference, or spread, between perception and

[2] The statistical analysis of the data was done by Dr. K. J. Arnold and associates in the Department of Mathematics, University of Wisconsin, Madison, Wisconsin.

TABLE 2

SUMMARY OF DATA ON TUBERCULOUS PATIENTS

PATIENT	AGE	EXTENT OF TUBERCULOSIS	DURATION OF TUBERCULOSIS*	NEUROPSYCHIATRIC DIAGNOSIS	PAIN PERCEPTION THRESHOLD	PAIN REACTION THRESHOLD
					gm. cal./sec./cm.^{2}	gm. cal./sec./cm.^{2}
MCN	27	Minimal	3 months	0	0.288	0.520
ABW	56	Minimal	6 months	0	0.404	0.555
EA	57	Far advanced	1 month	0	0.326	0.480
JK	20	Far advanced	12 months	0	0.288	0.326
RWN	29	Moderately advanced	3 months	0	0.365	0.555
JAP	29	Minimal	3 months	0	0.288	0.365
RJF	30	Moderately advanced	6 months	0	0.365	0.555
JEB	33	Minimal	9 months	0	0.365	0.555
JEW	24	Minimal	9 months	0	0.252	0.365
DEW	29	Minimal	6 years	0	0.326	0.404
TFMc	25	Moderately advanced	6 months	0	0.252	0.442
SW	24	Moderately advanced	12 months	0	0.252	0.442
TDJ	25	Moderately advanced	6 months	0	0.288	0.520
LKP	35	Moderately advanced	6 years	0	0.252	0.404
FPA	57	Far advanced	4 years	0	0.326	0.442
JFC	57	Far advanced	18 months	0	0.288	0.480
EJD	50	Far advanced	2 years	0	0.365	0.555
WF	33	Moderately advanced	3 months	Character disorder with chronic alcoholism	0.326	0.365
TJF	57	Far advanced	18 months	Character disorder with chronic alcoholism	0.326	0.442
EWT	45	Far advanced	12 months	Character disorder with chronic alcoholism	0.252	0.326
MCC	40	Moderately advanced	6 months	Character disorder with chronic alcoholism	0.326	0.404
AM	41	Far advanced	18 months	Character disorder with chronic alcoholism	0.252	0.404
PEW	56	Moderately advanced	3 months	Character disorder with chronic alcoholism	0.326	0.480
CE	57	Minimal	3 months	Psychoneurotic reaction, mixed	0.252	0.480
FMD	27	Minimal	6 months	Psychoneurotic reaction, mixed	0.288	0.442
HL	57	Moderately advanced	3 months	Multiple sclerosis	0.288	0.520
WRJ	30	Moderately advanced	4 years	Anxiety reaction	0.288	0.288†
WNH	41	Far advanced	3 months	Anxiety reaction	0.326	0.326†
HKR	24	Minimal	9 months	Anxiety reaction	0.288	0.288†
RRR	26	Minimal	3 months	Anxiety reaction	0.288	0.288†

* Time since positive tuberculosis diagnosis made.

† Fusion of pain perception and reaction thresholds.

reaction thresholds in the patients with neuropsychiatric diagnoses was significantly reduced as compared with the mean difference between the perception and reaction values in the tuberculous patients without NP diagnoses (table 3).

TABLE 3

PAIN THRESHOLDS OF TUBERCULOUS PATIENTS WITH AND WITHOUT NEUROPSYCHIATRIC DIAGNOSES

GROUP	WITH NEUROPSYCHIATRIC DIAGNOSES	WITHOUT NEUROPSYCHIATRIC DIAGNOSES
Number of patients	13	17
Pain perception threshold (mean) in gm. cal./sec./cm.^{2}	0.294	0.311
Pain reaction threshold (mean) in gm. cal./sec./cm.^{2}	0.388*	0.468
Difference in means in gm. cal./sec./cm.^{2}	0.094*	0.157

* Significant at the 5 per cent level of significance.

The mean pain reaction threshold and the mean difference, or spread, between perception and reaction thresholds of the neuropsychiatric group show a statistically significant reduction in comparison to the non-neuropsychiatric group.

TABLE 4

PAIN THRESHOLDS OF TUBERCULOUS PATIENTS CLASSIFIED ACCORDING TO EXTENT OF PULMONARY DISEASE

GROUP	NUMBER OF PATIENTS	PAIN PERCEPTION THRESHOLD (MEAN)	PAIN REACTION THRESHOLD (MEAN)
		gm. cal./sec./cm.^{2}	gm. cal./sec./cm.^{2}
Minimal	10	0.304	0.426
Moderately advanced	11	0.302	0.452
Far advanced	9	0.305	0.420

The differences in the means are not statistically significant.

TABLE 5

PAIN THRESHOLDS OF TUBERCULOUS PATIENTS CLASSIFIED ACCORDING TO DURATION OF PROVED TUBERCULOSIS

GROUP	NUMBER OF PATIENTS	PAIN PERCEPTION THRESHOLD (MEAN)	PAIN REACTION THRESHOLD (MEAN)
		gm. cal./sec./cm.^{2}	gm. cal./sec./cm.^{2}
Three months or less	10	0.307	0.414
Six to nine months	9	0.314	0.458
One to six years	11	0.292	0.430

The differences in the means are not statistically significant.

There were 4 instances of fusion of perception and reaction threshold. This phenomenon occurred in the 4 patients who had diagnoses of anxiety reaction (table 2).

Comparison of tuberculous patients on the basis of extent and duration of their pulmonary disease: When these data were analyzed in regard to extent of pulmo-

nary tuberculous disease, it was found that the patients were nearly equally divided into three categories: 10 had minimal disease, 11 had moderately advanced, and 9 had lesions which were classed as far advanced. The mean pain perception and reaction thresholds for these three groups were not found to be significantly different (table 4). Similarly, when classified on the basis of the proved duration of their pulmonary tuberculosis, the patients were about evenly distributed into three categories. Again, there was no statistically significant difference between these three groups in terms of mean pain perception and reaction thresholds (table 5).

DISCUSSION

It has been demonstrated in the present investigation that the pain thresholds of a group of tuberculous patients did not differ significantly from those of a comparable series of hospitalized general medical patients. Among the patients with tuberculosis, however, it was shown that the group with neuropsychiatric diagnoses had a statistically significant reduction in mean pain *reaction* threshold. In this regard, the tuberculous patients were similar to nontuberculous individuals who had emotionally conditioned disorders such as psychoneurosis, peptic ulcer, and the "menopausal syndrome" (3, 4, 5, 18, 19, 20).

Thus, the results of this experimental study support the more modern beliefs, derived from clinical observation and psychologic testing, regarding the occurrence of emotional disturbances in tuberculous patients. It is the current consensus: (*1*) that neuropsychiatric disorders are no more prevalent among the tuberculous than in the general population (2, 8, 9, 10), and (*2*) that the individual's basic personality traits and previous emotional stability are the critical factors determining the type and degree of emotional maladjustment manifested by the patient ill with tuberculosis (6, 7, 8, 10, 12, 13, 16, 17, 21).

The finding that there was no positive correlation between extent or duration of pulmonary disease and altered pain response in the tuberculous patients in this series is also in keeping with the impressions gained from studies in which other means of measurement or evaluation of emotional factors were utilized (10, 16, 17, 21). These results are quite opposed to such long-held notions as "spes phthisica" (1), the indifferent or euphoric attitude ascribed to patients with far advanced tuberculosis, and "toxins" (15) of *M. tuberculosis* which have been regarded as causative of mental and emotional aberrations in the tuberculous.

Besides the foregoing, this study demonstrates one way in which Berle's recommendation (1) that experimental methods involving "measurable physiologic changes" may be applied in the investigation of emotions and bodily changes in tuberculosis.

SUMMARY

Using the Hardy-Wolff-Goodell pain threshold apparatus as a means of demonstrating the hyper-reactivity to noxious stimuli found to occur in emotionally disturbed individuals, 30 tuberculous veterans and 30 male medical patients hospitalized with other illnesses were tested. The results were then analyzed

statistically for the purpose of comparing the two groups in terms of pain thresholds. Moreover, the findings in the tuberculous subjects were correlated with such factors as mental status and extent and duration of the pulmonary disease.

It was found: (*a*) that the mean pain thresholds of the tuberculous patients were not significantly different from those of the general medical patients; (*b*) that among the patients with tuberculosis it was only the group with neuropsychiatric diagnoses which had a significant reduction in pain *reaction* threshold; and (*c*) that no positive correlation existed between pain thresholds and the extent or duration of pulmonary tuberculosis.

It is concluded that these data offer some experimental support to the modern belief that it is the "person," rather than the pulmonary disease per se, which is the critical factor determining the occurrence of emotional disturbances in patients who develop tuberculosis.

SUMARIO

El Umbral del Dolor, las Emociones y la Tuberculosis

Usando el aparato de Hardy-Wolff-Goodall dedicado a determinar el punto umbral del dolor como medio de descubrir la hiperreactividad a los estímulos nocivos presente en los individuos perturbados afectivamente, tratóse a 30 veteranos tuberculosos y a 30 sujetos varones hospitalizados con otras enfermedades. Los resultados fueron luego analizados estadísticamente a fin de comparar esos dos grupos en términos de umbral del dolor y de correlacionar los hallazgos en los tuberculosos con factores tales como estado psíquico y extensión y duración de la afección pulmonar.

Observóse: (*a*) que los puntos medios de umbral del dolor en los tuberculosos no discrepaban significativamente de los observados en los casos de medicina general; (*b*) que entre los tuberculosos sólo el grupo con diagnósticos neuropsiquiátricos mostraba disminución significativa en el umbral de reacción al dolor, y (*c*) que no existía correlación positiva entre los umbrales del dolor y la extensión o duración de la tuberculosis pulmonar.

Dedúcese que esos datos ofrecen algún apoyo experimental a la moderna creencia de que la "persona," más bien que la enfermedad pulmonar en sí misma, constituye el factor crítico que determina la ocurrencia de trastornos emotivos en los enfermos que manifiestan tuberculosis.

RESUME

Seuil de la douleur, émotions et tuberculose

L'appareillage Hardy-Wolff-Goodel pour la mesure du seuil de la douleur a été utilisé pour démontrer l'hyper-réactivité à un stimulus nocif manifestée par les sujets affectés de troubles émotionnels; 30 vétérans tuberculeux et 30 malades hospitalisés pour d'autres affections ont été étudiés. Les résultats ont été soumis à l'analyse statistique dans le but de comparer ces deux groupes en termes de seuil de la douleur et d'établir un rapport entre les résultats obtenus chez le tuberculeux et certains facteurs, tels que l'état psychique et l'étendue et la durée de l'affection pulmonaire.

On a trouvé: (*a*) que les seuils moyens de la douleur, obtenus dans la tuberculose ne différaient pas de façon significative de ceux obtenus dans les cas de médecine générale; (*b*) que, dans le groupe des tuberculeux, seuls, ceux pour lesquels un diagnostic neuropsychiatrique avait été posé montraient une réduction significative du seuil de la réaction à la douleur; (*c*) qu'il n'y avait aucune corrélation positive entre le seuil de la douleur et l'étendue ou la durée de la tuberculose pulmonaire.

Les auteurs concluent en indiquant que ces données expérimentales viennent à l'appui du concept moderne que "l'individu" plutôt que l'affection pulmonaire en soi, représente le

facteur critique déterminant l'appartition de troubles émotionnels chez le sujet qui devient tuberculeux.

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BRONCHIECTASIS AS SEEN IN AN AMBULANT CLINIC SERVICE[1]

A Follow-up Study of Forty-nine Cases over a Minimum Period of Nine Years

ANSON McKIM[2]

(Received for publication May 26, 1952)

Introduction

Bronchiectasis is described by most writers as a progressive debilitating disease which may be expected to terminate fatally. Surgical removal of the diseased lung tissue is generally believed to be the only means of rescuing the patient from chronic invalidism or premature death. That this is the prevailing concept may be exemplified by reference to standard textbooks. Lisa and Rosenblatt (1) describe the usual course of bronchiectasis as "one of recurring pulmonary exacerbations . . . . eventually terminating in death," while Coope (2) pictures the patient as "at best a chronic invalid, a misery to himself and an offense to others." As stated by Muschenheim in Cecil-Loeb's *Textbook of Medicine* (3), the average duration of life from the onset of symptoms has generally been estimated to be ten to fifteen years.

On the other hand, the frequent discovery of a seemingly benign type of bronchiectasis has been reported by a number of investigators in the past ten years (4–9). A review of the literature, however, fails to reveal any follow-up studies of such patients. It is not clear, therefore, whether these apparently benign cases remain benign or eventually develop the classical symptoms and complications of bronchiectasis.

From Ray Brook State Tuberculosis Hospital, "Consultation Chest Clinics" have been conducted in the surrounding counties of New York State's Adirondack area for more than twenty-five years. The discovery of bronchiectasis among patients referred to these clinics has not been unusual and, as a rule, such patients have returned year after year for re-examination. Because these patients rarely exhibited evidence of progressive disease, physicians in the clinic service have long been of the opinion that bronchiectasis, as encountered under such circumstances, has little resemblance to the disease described in medical textbooks. The present investigation was undertaken in order to confirm or disprove this clinical impression and to investigate the natural course of bronchiectasis in a group of patients drawn from an ambulant clinic service.

Clinical Material and Methods of Study

Selection of cases: All of the group studied were ambulant clinic patients although a few had been admitted to the hospital from the clinic service because tuberculosis was suspected.

To obtain a group of patients for study, all bronchograms done between 1930 and 1941 were reviewed, and the cases in which bronchiectasis was demonstrated were set aside for

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further analysis. The study was restricted to cases diagnosed in the 1930 to 1941 interval because it was not possible to obtain all of the bronchograms done earlier than 1930 and because it was believed that a minimal follow-up period of nine years was preferable to a shorter follow-up study of a larger group. Excluded from the study were patients who also had significant pulmonary tuberculosis or pulmonary cancer and two patients treated by lobectomy. Etiologic factors, other than the above, were not considered reasons for exclusion, i.e., foreign-body inhalation or acute lung abscess.

Follow-up methods: Of 53 cases thus collected, 49 patients were located and their condition determined. On the basis of incomplete information, there was no reason to believe that the other 4 were not alive. Thirty-three of the surviving patients were seen in a chest clinic for the final follow-up examination, chest roentgenograms were obtained, and in 8 instances bronchographic examination was repeated. For the remaining 8 surviving patients, questionnaires answered by private physicians or public health nurses and recent roentgenograms taken elsewhere provided the necessary information. The majority of the group studied have been examined periodically throughout the nine- to twenty-year follow-up period in our hospital clinics.

TABLE 1

TYPE AND EXTENT OF BRONCHIAL DILATATION AS DEMONSTRATED BY INITIAL BRONCHOGRAMS

EXTENT	CYLINDRICAL	SACCULAR	TOTAL
Incipient	3	0	3
Mild	10	0	10
Moderate	13	13	26
Extensive	2	8	10
Total	28	21	49

Sex and age: The group studied consisted of 27 females and 22 males. The average age at the estimated onset of symptoms was twenty-two years and the average age at the time of bronchographic diagnosis was thirty-one years. Ages at the time of diagnosis ranged from five to sixty-four years.

Bronchographic findings: After reviewing the original bronchograms, all cases were classified according to the type and extent of bronchiectasis.

The classification used was as follows:

Type

(1) cylindrical
(2) saccular

Extent

(1) incipient (slight dilatation of one or two bronchi)
(2) mild (definite ectasia localized to one or two segments in one lobe)
(3) moderate (extensive ectasia of one lobe, or ectasia in two lobes not extensive in either)
(4) Extensive (more than moderate in extent)

By the above system, the 49 cases have been classified in table 1. It will be seen that most cases were of moderate extent. The earlier bronchographic studies were often limited to one lobe or one lung, and other areas of bronchiectasis may often have been present, although not demonstrated. It is probable, therefore, that these patients had more extensive disease than is indicated by the table.

Duration of symptoms: Even when a detailed history is available, it is often difficult to decide when the symptoms of bronchiectasis began. Estimates of the duration of symptoms

represent a minimal figure, for suspicious evidence of bronchiectasis had often appeared long before the development of symptoms or complications that warranted a positive diagnosis. In 2 instances no time of onset could be estimated, but the remaining 47 patients had suffered from symptoms attributable to bronchiectasis for an average of 8.5 years before diagnosis was made by bronchography; this interval varied from a few months to 36 years.

Results

Fatal cases

In table 2 are summarized the pertinent facts concerning the 8 patients who died during the follow-up period.

TABLE 2

Descriptive Summary of the Findings in 8 Patients Who Died During the Follow-up Period

Immediate cause of death	Age at death	Extent of ectasia*	Symptoms*	Years from diagnosis to death
Deaths attributable to bronchiectasis:				
Acute pulmonary abscess	28	Extensive	Severe	6
Osteomyelitis, multiple abscesses	20	Extensive	Severe	3
Right-sided heart failure	59	Extensive	Severe	7
Unknown (progressive bronchiectasis)	18	Extensive	Severe	5
Acute bronchopneumonia	49	Moderate	Moderate	2
Deaths not attributable to bronchiectasis:				
Coronary disease and hypertension	74	Moderate	Moderate	10
Left-sided heart failure	55	Moderate	Moderate	8
Bronchogenic carcinoma	60	Moderate	Moderate	9

* As noted at the time of diagnosis.

It will be seen that 5 deaths were due to bronchiectasis or its complications and the other 3 to unrelated diseases. Thus, the total mortality is 16.3 per cent, but the mortality attributable to bronchiectasis is only 10.2 per cent. The table reveals notable differences between the two groups in the age at death, the severity of symptoms, the extent of bronchiectasis and, to a lesser degree, in the interval from diagnosis to death.

Living cases

Forty-one patients were still alive 9 to 18 years after the diagnosis of bronchiectasis. The average follow-up period for living patients was 12.5 years, varying from 9 to 20 years, and the average duration of symptoms at the end of the study was 21.5 years.

Cough and expectoration: The symptom complex of cough and expectoration was graded as mild, moderate, or severe on the basis of the amount of sputum raised. Eight cubic centimeters of sputum or less per day were considered mild;

60 cc. or less, moderate; and more than 60 cc., severe. The degree of cough and expectoration at the beginning and the end of the follow-up period is demonstrated in table 3. There was a fairly close correlation between the extent of bronchial ectasia and the severity of symptoms. The table illustrates the general improvement that occurred. Further analysis of the follow-up findings showed that at the final examination 26 patients were raising less, 12 were raising the same amount, and only 3 were raising more sputum than had been recorded at the time of diagnosis.

Symptomatic course: In table 4 the follow-up status of living patients with regard to all respiratory or constitutional symptoms is presented. In determining whether symptoms had increased, decreased, or remained the same over

TABLE 3

COMPARISON OF THE DEGREE OF COUGH AND EXPECTORATION AMONG SURVIVING PATIENTS AT THE TIME OF DIAGNOSIS AND AT THE END OF THE FOLLOW-UP STUDY

	NONE	MILD	MODERATE	SEVERE	TOTAL
At the time of diagnosis	1	13	14	13	41
At the end of the study	5	23	8	5	41

TABLE 4

THE SYMPTOMATIC COURSE AMONG SURVIVING PATIENTS DURING THE FOLLOW-UP PERIOD

	CYLINDRICAL	SACCULAR	TOTAL
Improved	16	6	22
Unchanged	8	8	16
Worse	2	1	3
Total	26	15	41

the follow-up period, it was possible to compare the patient's own opinion with the clinical reports made at the time of diagnosis and at the final examination. In most cases the course of the disease could be followed in detail by reviewing periodic clinic reports. The reference is only to chronic symptoms; recovery from an acute illness present at the time of diagnosis was not considered an improvement.

It may be seen that patients with cylindrical bronchiectasis did better than those with the saccular type, as might be expected. During the nine- to eighteen-year interval, symptoms had become more severe in only 6 per cent of surviving patients; 54 per cent experienced an amelioration of symptoms; and in 40 per cent there had been no change. Many patients improved markedly over the years. For example, 7 patients who had previously raised foul sputum ceased to do so, and 4 patients claimed that their chronic cough had completely disappeared.

Physical incapacity: It is often difficult to decide whether or not a person

suffers from physical incapacity and even more difficult to relate such incapacity to a single disease process. In this study, patients were considered to be physically incapacitated if they had found it necessary to limit their activities or change their occupations or places of residence because of frequent illness or chronic symptoms, respiratory or otherwise. Due allowance was made for natural incapacity due to aging.

In table 5 is illustrated the degree of incapacity existing in the group of surviving patients. One case has been omitted because of inadequate information. Seventy-five per cent of the living patients had suffered no physical incapacity, and the extent of the bronchiectasis seemed to have little influence in this respect. Further study of the 10 partially incapacitated patients showed that the youngest was thirty-eight years of age, 7 of the 10 were more than

TABLE 5

THE DEGREE OF PHYSICAL INCAPACITY AMONG SURVIVING PATIENTS AT THE END OF THE FOLLOW-UP PERIOD, REGARDLESS OF CAUSE

DEGREE OF PHYSICAL INCAPACITY	EXTENT OF DISEASE			TOTAL
	Incipient and Mild	Moderate	Extensive	
None	10	16	4	30
Partial	3	5	2	10
Complete	0	0	0	0
Total	13	21	6	40

fifty years of age, and 4 were older than sixty. The incapacity of 4 patients probably had little or no relationship to their bronchiectasis, other unrelated disease being present.

SELECTION OF SURVIVORS

It was believed that those patients who gave a long history of symptoms would to some extent represent "survivors" whose contemporaries suffering from more severe diseases had died, thus creating a selected group. Estimating the date of symptom onset as accurately as possible, it was found that 22 patients had symptoms for five years or less before diagnosis, while 25 patients had symptoms for six years or more. In 2 instances no estimates could be made. Thus, approximately half of the group had disease of relatively recent onset.

Table 6 was prepared in an attempt to determine whether such "survivors" fared differently from patients with more recent disease. The table includes only those patients who gave an unequivocal history of symptoms beginning either less than six, or more than ten, years prior to diagnosis. Fortuitiously, 17 patients fell into each group. Contrary to what might have been expected, the "survivors" did not fare as well as the other group. However, although improvement was less frequent among patients with a long history, symptoms were not more severe. The lack of improvement appeared to represent only a

greater degree of stability. There was also more incapacity among the "survivors" group, but they were, on the average, ten years older. There were several children in the group with a short history of symptoms prior to diagnosis. No children were included in the other group. Children, if they did not die of the disease, appeared to be more liable to improve and regain good health than did adults.

COMPLICATIONS

"Pneumonia": Recurrent attacks of pneumonia are considered to be a common complication of bronchiectasis, but only 11 of the patients had one or more attacks of pneumonia during the follow-up period. Two of the 11 were among the fatal cases. It is probable that the other patients who died also suffered attacks of pneumonia, for in these instances very little information was available in regard to the course of the disease after diagnosis.

Of the 9 living patients who gave an interval history of pneumonia, only 2 had more than one attack. Primary atypical pneumonia, postoperative pneumonia, or any acute illness characterized by prolonged fever and a marked increase of respiratory symptoms was considered as an attack of "pneumonia." No doubt some patients suffered unrecognized and, therefore, unrecorded attacks of pneumonia but, even when allowances are made for such errors, this complication was surprisingly rare.

TABLE 6

A Comparison of the Follow-up Findings Among a Group of Patients with Bronchiectasis of Recent Onset as Opposed to the Findings in a Similar Group Who Gave a Long History of Symptoms Prior to Diagnosis

Interval from onset of symptoms to diagnosis	Dead	Living	Respiratory symptoms			Incapacity	
			Worse	Unchanged	Improved	Partial	None
0 to 5 years (17 cases)	3	14	0	1	13	0	14
More than 10 years (17 cases)	2	15	1	7	7	7	8

Colds: Of 39 living patients with an adequate history, 17 (43.5 per cent) admitted that they suffered from repeated prolonged or febrile colds.

Hemoptysis: While "blood-spitting" had occurred in almost all cases at some time, many patients had experienced only blood-streaking of their sputum, and most frank hemoptysis (at least 4 cc. of blood) had occurred at the time of the onset of symptoms. Only 8 surviving patients gave a history of hemoptysis during the follow-up period and in none of these was the bleeding copious. Hemoptysis was much more frequent among fatal cases, and large hemorrhages were not uncommon.

Foul sputum: Eleven of the entire group had raised foul or fetid sputum at some time. Four of these died. The 7 survivors who raised foul sputum did so only at the acute onset of their symptoms or intermittently when suffering from acute respiratory infections. Only one living patient had noted foul sputum in recent years.

Other complications: Other conditions, possibly attributable to bronchiectasis, consisted of one retinal hemorrhage, one instance of persistent albuminuria (without other evidence of amyloid disease), and one instance of recurrent otitis media. One patient who died may have had a brain abscess, and another patient died of an acute lung abscess.

DIAGNOSTIC SIGNS

Clubbing of the fingers: Two of the patients who died had clubbed fingers. The presence or absence of this sign had not been recorded in the available reports of other fatal cases. Of the living patients, only one had clubbed fingers at diagnosis, but the sign was absent at the final follow-up examination. This patient has had severe cough and expectoration, associated with extensive saccular bronchiectasis, for thirty-six years.

TABLE 7

ABNORMAL SIGNS DETECTED BY AUSCULTATION OF THE LUNGS AT THE BEGINNING AND AT THE END OF THE FOLLOW-UP STUDY (SURVIVING PATIENTS ONLY)

PHYSICAL SIGNS	AT THE TIME OF DIAGNOSIS	AT THE FINAL EXAMINATION
Moist fine rales	38	21
Rhonchi or abnormal breath sounds only	1	6
No abnormal signs	2	7
Not examined	0	7
Total	41	41

Pulmonary physical signs: In table 7 are summarized the findings on auscultation of the lungs (*a*) at the time of the bronchographic diagnosis and (*b*) at the end of the follow-up study. All patients were examined at the time of bronchographic diagnosis; 7 surviving patients were not examined at the end of the study. Fatal cases are not included in the table; in all of these, abnormal physical signs were present. It will be seen that physical signs were rarely absent at the time of diagnosis and that they persisted, as a rule, throughout the follow-up period. This was true regardless of the extent of the bronchiectasis. In all 3 "incipient" cases, abnormal signs were present at the time of diagnosis and, in 2 of these patients, rales were present eleven years later. Of 9 patients with mild disease, all had abnormal signs at diagnosis which, in all but 2 instances, persisted for nine to seventeen years. It would seem that, for practical purposes, the most valuable instrument in discovering bronchiectasis is the stethescope rather than the X-ray machine.

Routine chest roentgenograms: At the time of bronchographic diagnosis, abnormal roentgenographic findings were present in all of the subsequently fatal cases. In table 8 are summarized the findings in the routine chest roentgenograms at the beginning and at the end of the follow-up period among living patients. It is apparent that in most patients evidence of pulmonary disease was exhibited

at the beginning of the study and that these abnormalities were usually permanent. Comparative study of the earliest and latest films of each patient revealed that the roentgenographic abnormalities of 9 had cleared partially, while in 5 the abnormalities had increased. The films of the remaining 22 patients did not change materially.

Repeated bronchograms: Eight patients had more than one bronchogram done at Ray Brook, the second study being performed two to eighteen years after the first. Six repeated studies showed no change. In 2 patients, repeated studies demonstrated an extension of the bronchial dilatation in contiguous areas over periods of two and eighteen years (Cases 3, 7, and 8).

TABLE 8

THE FINDINGS ON ROUTINE CHEST ROENTGENOGRAMS AT THE BEGINNING AND AT THE END OF THE FOLLOW-UP PERIOD (SURVIVING PATIENTS ONLY)

CHEST ROENTGENOGRAMS	AT THE TIME OF DIAGNOSIS	AT THE FINAL EXAMINATION
Abnormal	31	27
Transient abnormalities only	3	0
Normal	7	9
Not taken	0	5
Total	41	41

RELATIONSHIP OF BRONCHIECTASIS TO PRIMARY TUBERCULOSIS

Primary tuberculosis in children may produce asymptomatic bronchiectasis (4, 5, 6). However, among 30 patients of this group, 17 were negative reactors to tuberculin. This corresponds closely enough with the over-all proportion of negative tuberculin reactors seen in these clinics (10) to warrant the conclusion that primary tuberculosis was not a frequent cause of bronchiectasis among the patients studied.

COMPARISON OF SURVIVING AND FATAL CASES

A marked difference could be noted on comparing in retrospect the manifestations of the disease at the time of diagnosis and the subsequent clinical course of the surviving as opposed to the fatal cases. Of the 5 patients who died of bronchiectasis or its complications, 4 had extensive saccular disease, severe symptoms, and were raising foul sputum. These 4 patients appeared chronically ill and poorly nourished at the time of diagnosis. The one exception was a patient with moderately extensive disease with moderate symptoms who appeared to be in good health. She died suddenly of acute bronchopneumonia two years later. After the diagnosis of bronchiectasis, at least 3 of the patients who subsequently died were admitted to hospitals because of severe respiratory illness on one or more occasions prior to the fatal episode.

Among surviving patients, many were suffering from an acute respiratory illness, or had recently recovered from such, at the time of diagnosis; but very

few appeared chronically ill. Living patients had rarely been admitted to hospitals for relevant illness during the follow-up period. The relatively infrequent incidence of pneumonia, hemoptysis, clubbed fingers, and foul sputum among surviving patients has already been described.

ILLUSTRATIVE CASE REPORTS

Death Not Due to Bronchiectasis

Case 1: A 41-year-old American Indian male was found to have bronchiectasis associated with collapse of the right lower lobe in 1939. There was also clinical and roentgenographic evidence of cardiac disease with left ventricular hypertrophy (figure 1). Respiratory symptoms, mild in degree and more suggestive of cardiac than pulmonary disease, had begun insidiously about 1935. The subsequent clinical picture was one of cardiac disease only. He died in 1947 from left-sided heart failure.

Progressive, Nonfatal Bronchiectasis

Case 2: The patient was a woman who had suffered from several acute respiratory illnesses as a child and adolescent. Nevertheless, chronic respiratory symptoms were noted only after an attack of pneumonia at the age of forty-one years. Bronchiectasis was demonstrated when she was fifty-six years of age (1936), the bronchogram revealing saccular and cylindrical dilatations in the right middle and lower, and the left lower, lobes (figure 2). At this time, although she raised 90 cc. of sputum daily, she was in good general health. There was evidence of pulmonary disease by physical examination, however, and on the routine chest roentgenograms.

The patient was re-examined at fairly regular intervals until 1950. At the last examination she was seventy-one years old and still appeared to be in fairly good general health. She believed she was as active as the average woman of her age, but admitted that she suffered from severe "colds" two or three times each year. Other complicating illnesses had not occurred during the fourteen-year follow-up period, but serial roentgenograms revealed evidence of gradually increasing "fibrosis" and emphysema, and abnormal physical signs had become much more extensive.

Nonprogressive Unimproved Bronchiectasis

Case 3: At the age of 5 years, the patient aspirated a screw. "Pneumonia" developed, and the screw was removed through a bronchoscope two months later. In June, 1929, nine months after the aspiration, he was first seen in Ray Brook clinic. Complaints were of recurrent "colds" and a persistent severe cough. Rales were heard over the left lower lobe area, and abnormal shadows were present in this area on routine chest roentgenograms. For the next six years, the patient was examined at yearly intervals with no significant change in his condition. In 1935, bronchographic study demonstrated marked saccular bronchiectasis confined to the left lower lobe (figure 3).

The patient was not seen again in the clinic until 1947, at the age of twenty-four years. His symptoms had improved, and the only complaint at this time was of a moderate cough productive of 15 cc. of sputum per day. He had been working steadily and had enlisted in the Army in 1943 but was discharged six months later when he developed "pneumonia." Bronchographic examination was repeated in 1947; all lobes were studied and it was concluded that no change had occurred in the type or extent of the bronchial dilatations since 1935 (figure 4).

When last examined in 1950, his symptoms remained the same. "Colds" occurred three or four times a year, but never lasted more than one week. He denied any respiratory symptoms except for a chronic cough productive of 15 cc. of sputum per day, which increased to 60 cc. during "colds." The pneumonia contracted during Army service had been

the only complication of his bronchiectasis during the entire follow-up period. Physical signs and routine chest roentgenograms were the same as in 1929. Treatment by lobectomy has been recommended for this patient on several occasions since 1947.

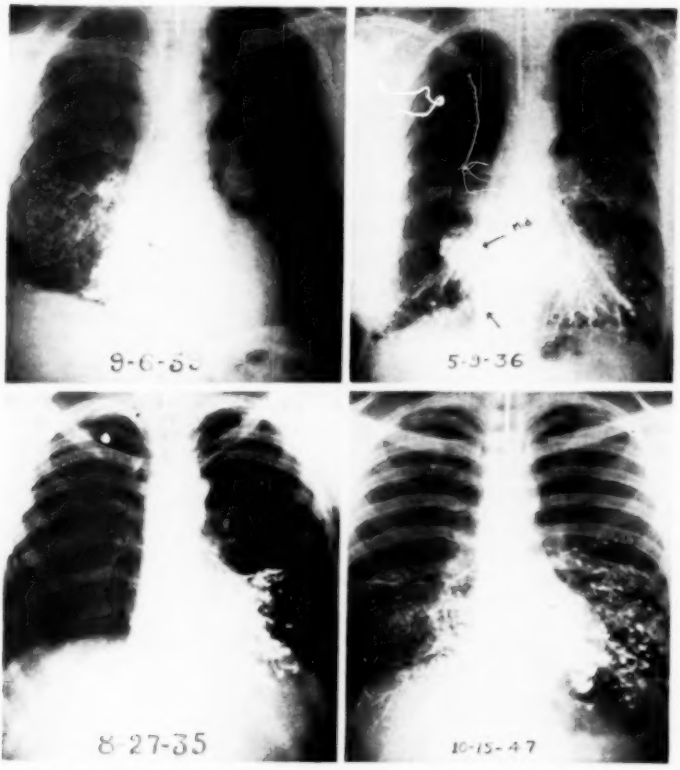

FIG. 1. *Case 1* (Upper left) Bronchogram demonstrating bronchiectasis in a shrunken left lower lobe. Note also enlargement of the heart.

FIG. 2. *Case 2* (Upper right) Bronchogram showing bronchial ectasia of at least three lobes (right and left lower and right middle), with partial collapse of right middle lobe.

FIG. 3. *Case 3* (Lower left) Marked saccular bronchiectasis of the right lower lobe. Bronchogram of 1935, patient 12 years of age.

FIG. 4. *Case 3* (Lower right) Repeat bronchogram of the same patient in 1947, age 24 years. There has been no significant change.

Case 4: A white female, whose symptoms began after an attack of pneumonia at the age of sixteen years, was first seen in the clinic at the age of twenty-eight (1938). She had a chronic cough of moderate degree; but with acute respiratory infections, which occurred frequently, she would raise as much as 480 cc. of sputum daily. Routine roentgenographic and physical examination revealed evidence of bilateral pulmonary disease. Bronchographic examina-

tion in 1940 demonstrated cylindrical bronchiectasis throughout the left lower lobe and of one or two bronchi in the right lower lobe (figure 5).

The patient was examined frequently between 1940 and 1950. There was gradual symptomatic improvement although a moderate chronic cough persisted. She continued to

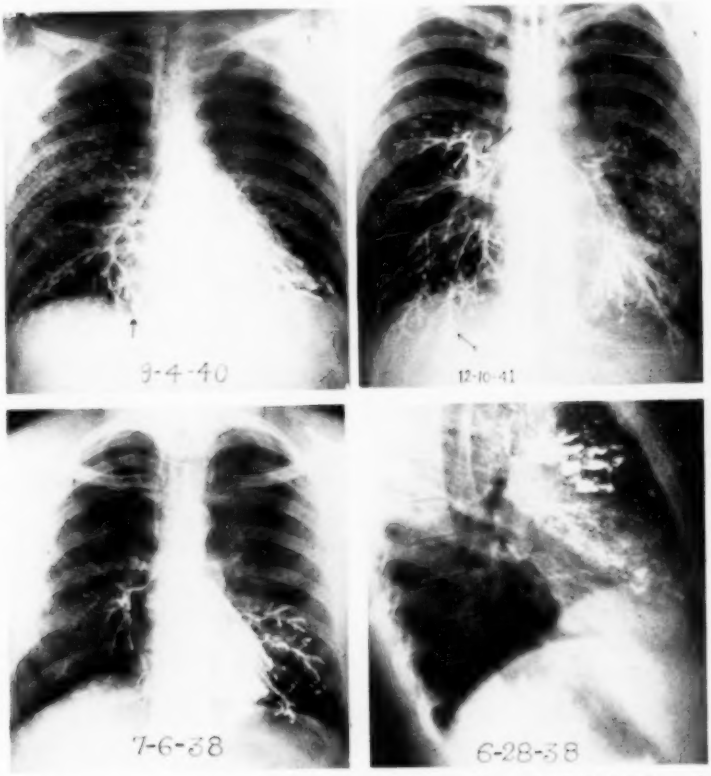

FIG. 5. *Case 4* (Upper left) Extensive cylindrical bronchiectasis of the left lower lobe and ectasia in one segment of the right lower lobe.

FIG. 6. *Case 5* (Upper right) A composite bronchogram demonstrating ectasia in three lobes: right upper and lower and left lower.

FIGS. 7 and 8. *Case 6* (Lower left and right) Posterior-anterior and right lateral bronchograms showing extensive bronchiectasis of the left lower and right upper lobes.

suffer from three or four prolonged "chest colds" each year and with these colds the quantity of sputum raised would increase from 15 to 120 cc. per day. More severe illnesses did not occur and she missed very little time at her work. At the final examination the patient appeared to be in good health, but rales and roentgenographic abnormalities were present as in 1940.

Clinically Improved Bronchiectasis

Case 5: A 28-year-old white male was first seen in the clinic in 1941. He gave a history of chronic productive cough and frequent chest colds with severe cough, expectoration, and wheezing; all present for "many years." Because of persistent rales and abnormalities of the chest roentgenogram, a bronchographic study was done which revealed extensive cylindrical bronchiectasis of both lower lobes and of the right upper lobe (figure 6).

The patient was not seen again in the clinic, but in 1950 a medical report and a chest roentgenogram were obtained from the staff physician at a prison where he had been employed as a guard for several years. His symptoms improved after 1941, and in recent years he had not had a chronic cough or other respiratory symptoms. There had been no incapacitating illnesses during the follow-up period, and he had not had a severe cold "for years." The patient believed that his general health had improved markedly over the previous nine years. He had enlisted in the Army during World War II, apparently passing medical examinations without detection of his bronchial disease. The results of a recent physical examination were not reported, but a chest roentgenogram of May, 1950, revealed no abnormalities.

Case 6: A 29-year-old well-developed and well-nourished white male was admitted to the hospital in 1938. The patient's symptoms had begun insidiously in 1931 and had gradually become worse. He complained on admission of a moderate cough productive of 30 cc. of sputum a day and of recurrent hemoptyses. Examination of the lungs revealed abnormal signs bilaterally, and "honeycombed" shadows were seen in the right upper chest on the routine chest roentgenogram. Complete bronchographic study in 1938 demonstrated extensive bilateral bronchiectasis which was saccular in the right upper lobe and cylindrical in the left lower lobe (figures 7 and 8). The patient was discharged "improved" in 1940. He went to New York City to live, but immediately experienced an aggravation of his symptoms and suffered several hemoptyses, which prompted his return to this hospital as an employee in 1941.

In 1951, after ten years as a full-time employee, he had been away from work because of illness for only five days. Colds occurred infrequently and they were never febrile or prolonged. The amount of sputum raised was less than 8 cc. per day, and he had not had a hemoptysis since 1941. As a waiter his work called for moderate exertion, but this did not cause dyspnea, in spite of lameness due to a postoperative neurologic disorder acquired as a youth. Physical examination revealed abnormal findings which had not changed over the previous ten years, and chest roentgenograms had changed only through the disappearance of a shadow previously present in the right lower lung.

Clinically Improved Bronchiectasis but Progressive by Bronchographic Study

Case 7: A 22-year-old white male, who gave no significant past history except for "frequent colds," was seen in the clinic in March, 1932. Two months before, he had developed a febrile illness with night sweats, weakness, anorexia, weight loss, and a severe cough productive of 240 cc. of thick sputum per day, which was frequently mixed with blood. He was described as a "sickly looking male" by the examining physician. Fetor oris was noted and rales were present over the left lower lobe. Routine chest roentgenograms appeared normal, but bronchograms revealed cylindrical dilatation of several bronchi in the retrocardiac portion of the left lower lobe (figure 9).

During the first year of the subsequent eighteen-year follow-up period, there was rapid symptomatic improvement. In 1936 his health appeared to be completely restored except for the persistence of a cough productive of approximately 120 cc. of nonfetid sputum daily. He worked steadily as a laborer and woodsman except for a period of service in the Army from 1944 to 1946, on limited duty because of "bronchitis."

When examined in September, 1950, at the age of thirty-nine, he was described as a "healthy looking muscular male." Auscultation of the lungs revealed no abnormalities, and

a routine chest roentgenogram was normal. Repeated bronchographic study in September, 1950 (eighteen years after the first bronchogram) revealed several large saccular dilatations

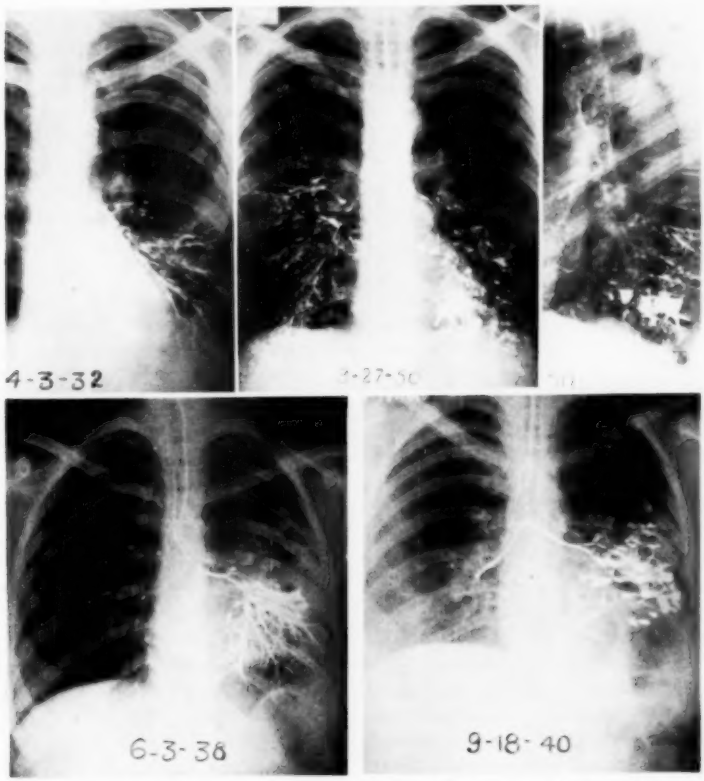

FIG. 9. *Case 7* (Upper left) Bronchogram of April, 1932, demonstrating bronchiectasis in the retrocardiac portion of the left lower lobe.

FIGS. 10 and 11. *Case 7* (Upper center and right) Posterior-anterior and left oblique bronchograms of the same patient 18 years later (September, 1950) showing localized extension of the bronchiectatic pockets.

FIG. 12. *Case 8* (Lower left) Bronchogram done in June, 1938. Bronchiectasis confined to the upper portion of the left lower lobe and the lingula.

FIG. 13. *Case 8.* (Lower right) Repeat bronchogram of the same patient two years later (September, 1940). All bronchi of the left lower lobe are now involved.

in the left lower lobe medially (figures 10 and 11). These had obviously developed at the site of the cylindrical dilatations seen on the bronchograms of 1932.

This patient was advised to have surgical treatment, and a left lower lobectomy was successfully performed at another hospital in 1951.

Clinically Improved Bronchiectasis Following Early Progression

Case 8: In 1928 this patient, then nine years of age, fractured several ribs in an accident. Apparently as a consequence, she developed a febrile respiratory illness and coughed up "blood and pus." Although she soon recovered from the acute episode, a chronic cough persisted.

The patient first came to the clinic at the age of nineteen because she had recently noted that her sputum became foul whenever she suffered from a cold. At this examination in 1938, she appeared undernourished, rales were present over the left lower lobe and lingula, and numerous dense abnormal shadows were seen at the left base on a chest roentgenogram. Bronchograms revealed large bronchial dilatations in the left lower lobe and lingula (figure 12).

During the next two years the patient suffered from frequent "severe colds." In 1941 bronchographic study was repeated and it was apparent that the ectasia, though still confined to the same lobes, had become more extensive. Large saccular dilatations had developed in bronchi which previously appeared to be normal (figure 13).

The patient then moved from the Ray Brook clinic district, but she was interviewed by a public health nurse for the present study. She believed that her health had improved after her last examination in 1941. She denied the existence of any physical incapacity or the presence of any symptoms except for a mild nonproductive cough. Severe colds had not occurred for "several years," and the only incapacitating illnesses during the follow-up period had been an attack of "virus pneumonia" and a pelvic operation. From the hospital where a tubo-ovarian abscess had been removed in 1950, it was learned that no disease aside from her pelvic condition had been detected, that the operation had been uneventful, and that she had been discharged in good health.

Discussion

The clinical findings in the majority of cases included in the present study do not correspond to the textbook descriptions of bronchiectasis, and the experience here reported does not conform to the experience of others who have published follow-up studies. It is believed that this discrepancy can readily be explained and that the explanation is of considerable importance.

Adequate follow-up studies of patients with bronchiectasis are few in number and, consequently, in all standard textbooks the same small group of original papers are cited as evidence of the poor prognosis in this disease. When these papers are read, it becomes apparent that they were all based on studies of patients seen on the wards of general hospitals in large cities. The following are the studies most frequently mentioned in bibliographies.

All were follow-up studies of medically treated patients over relatively short periods of time. The average follow-up was 4 to 8 years and ranged among individual patients from 1 to 16 years. Roles and Todd (11) in 1933, from the Brompton Hospital, London, reported a 47 per cent mortality rate. Perry and King (12) in 1940 found that 31 per cent of Massachusetts General Hospital patients were dead and 20 per cent of the survivors were in poor or very poor health. A mortality rate of 27 per cent was reported from the Royal Victoria Infirmary, Newcastle, England, by Ogilvie (13) in 1941. Also in 1941, Bradshaw and coworkers (14), at the Jefferson Medical College Hospital, reported a 34 per cent mortality. Most frequently quoted, however, is Bradshaw's observation that the duration of life after diagnosis, in fatal cases, was only 1.8 years. From Bellevue and Lenox Hill Hospitals, Riggins (15) reported only a 14 per cent death rate; but 75 per cent

of the surviving patients were unable to do full-time work and 35 per cent of these could not work at all.

Cookson and Mason (16) did not report a follow-up study, but their observations are frequently quoted. In a paper entitled, "Bronchiectasis—A Fatal Disease," they describe 21 cases to prove the validity of this title. It is to be noted, however, that all but one of these were autopsy cases, and that 12 of the 21 cases were diagnosed only at autopsy.

Patients with bronchiectasis who are admitted to the wards of general hospitals, such as those named above, represent a selected group, and studies of such patients will not give a true picture of the disease as it occurs in the general population. Cases discovered in ambulant clinics, such as those described in this study, seldom have severe symptoms and rarely develop complications which require hospitalization. However, the fact that bronchiectasis is associated with severe symptoms and complicated by recurrent acute illnesses in almost all cases seen on a hospital ward has led to the belief that benign forms of the disease are rare exceptions to the general rule (13, 15).

The experience with bronchiectasis of the present writer is not unique for it has become common practice in recent years to use bronchography as a diagnostic aid in the investigation of obscure pulmonary disease, and not merely as a means of confirming or clarifying a clinical diagnosis. This practice has demonstrated the presence of bronchiectasis in many patients who have only mild symptoms, atypical roentgenographic abnormalities, or unexplained physical signs.

In this way, Martin and Berridge (7) in 1942 discovered 25 cases of saccular and cylindrical bronchiectasis among Royal Air Force personnel in England. All had passed thorough medical examinations and were performing rigorous duties. Fine and Steinhausen (8) in 1946, under similar circumstances, found 41 cases of bronchiectasis among U. S. Army men. Despite the fact that bronchography often revealed extensive ectasia, none of these men admitted to more than mild chronic symptoms and many were asymptomatic. Evans and Galinsky (9) reported the discovery of 61 similar cases, also among apparently healthy U. S. Army servicemen.

Predominantly benign bronchiectasis also has been reported from hospitals serving civilians. Franklin (17) followed 24 children for four to twelve years; although one child had died and 4 were classed as invalids, 4 were described as "delicate" and 15 were "absolutely well." Wissler and Hotz (18) reported that 63 per cent of 38 traced patients were well and working six to fifteen years after diagnosis. In Cecil-Loeb's *Textbook of Medicine*, Muschenheim (3) states that "most published studies refer to patients with extensive involvement." He notes that patients with "limited disease" are frequently followed for five to ten years without apparent change.

Several recent reports (4, 5, 6) have described a benign type of bronchiectasis caused by primary tuberculosis in children, apparently the result of transient bronchial obstruction rather than parenchymal disease. Such ectasia may be extensive and indistinguishable from bronchiectasis of nontuberculous origin. It seems probable that this is a relatively common occurrence, but that chronic

or progressive suppuration does not persist after the resolution of the tuberculous process and, therefore, these children remain asymptomatic.

The present writer believes that bronchiectasis in its clinical course resembles chronic pulmonary tuberculosis in many ways and that a number of valid comparisons may be made. In both instances the disease varies from a progressive and rapidly fatal illness to a nonprogressive one which may give rise to no overt symptoms. Bronchiectasis, like tuberculosis, is not infrequently an unexpected finding at autopsy (19). In either disease, exacerbations and complications may occur frequently, rarely, or not at all, such clinical manifestations being in both instances related to the activity of the inflammatory process rather than to the anatomic extent of demonstrable lesions. Just as a diagnosis of pulmonary tuberculosis is not complete until modifying adjectives such as *active*, *arrested*, or *inactive* can be appended, bronchiectasis is not a satisfactory diagnosis without the addition of qualifying terms.

No one denies that patients obviously ill with pulmonary tuberculosis often recover, but it is frequently stated that bronchiectasis cannot be "cured" because the dilatations are irreversible (1, 2). There is a great difference, however, between uncomplicated bronchial dilatation and bronchial dilatation associated with suppuration; probably just as great and significant a difference as exists between active and inactive tuberculosis. If it be granted that benign bronchiectasis must once have been suppurative in type, the discovery of many benign cases among such groups as military personnel indicates that healing of the inflammatory component occurs rather frequently. The present study supports this inference, for a number of the patients who suffered from the classical symptoms of bronchiectasis at the time of diagnosis were almost asymptomatic and in good health a decade or more afterwards.

It is known that the mortality curve in pulmonary tuberculosis rises rapidly in the first few years after the onset of the disease and then rapidly levels off (20). It seems probable that a mortality curve for bronchiectasis would bear a close resemblance, for, as Bradshaw and associates (14) have pointed out, fatalities usually occur early in any follow-up period. Most investigators have taken pains to point out the variable course of bronchiectasis, but attention is usually focused on the proportion of fatal terminations when the results of a study are interpreted. It is often assumed, and not infrequently stated, that survivors will also succumb at an early date; therefore, they are subsequently ignored.

Many studies could be quoted to illustrate the considerable proportion of healthy survivors, even when the groups studied were drawn exclusively from hospitalized patients. Ford (21) followed children after discharge from a Glasgow hospital for two to nineteen years. Of 49 "medically-treated" cases, 13 died, 21 were alive but with some symptoms, and 15 were alive and asymptomatic. The study of Perry and King (12) was previously cited to illustrate the high mortality rate of hospitalized patients (31 per cent); however, among the survivors, 38 per cent were in "excellent," and 42 per cent in "fair," health. Similarly, Roles and Todd (11) reported a 47 per cent death rate, but 34 per cent of their survivors were well and in no way incapacitated. The above were all

follow-up studies of "medically treated" groups. One cannot estimate to what degree the milder cases were selected for surgical treatment, but certainly all of the extensive inoperable cases remained in these "medically treated" groups.

Discussion of the treatment of bronchiectasis is beyond the purpose of the present paper, but the results of the study, supported by the previously quoted findings of other investigators who have encountered benign bronchiectasis, prompt a few comments. The value of treatment by surgical excision, in saving lives and in making other lives more pleasant and productive, cannot be questioned. As a result of the present follow-up study, surgical treatment has been advised for several patients in spite of the fact that their disease has been clinically benign or nonprogressive during nine to eighteen years of observation. On the other hand, many writers in recent years, impressed by the success of lobectomy and pneumonectomy operations, have recommended surgical excision for virtually all cases when the necessary operation is possible (12, 15, 22).

It is believed that such a facile and undifferentiating approach to the treatment of bronchiectasis is entirely unjustified. If it be true that bronchiectasis, like pulmonary tuberculosis, is often a self-limiting condition, the former disease no less than the latter will require careful evaluation before surgical treatment can justifiably be undertaken or withheld. Lee Lander (23), after making observations similar to those of this study, has recommended that surgical treatment should not be considered until the patient has been observed for at least a year after a diagnosis of bronchiectasis is first made.

No doubt one may safely advise lobectomy for a young patient with localized bronchiectasis, even though manifestations of the disease are mild. But what should one recommend to the patient who is no longer young or to the individual with benign but multilobar or bilateral disease? The operative mortality rate for simple lobectomy is now very low, but apparently this is not true if pneumonectomy or bilateral excision is necessary. Streider (24) reported an operative mortality (case rate) of 27.6 per cent for pneumonectomies and 28.5 per cent for bilateral lobectomies in 1948. Lambert (25) in 1950 had a mortality rate of 28 per cent for pneumonectomies, but he considered most of these to be "salvage cases." Kergin's (26) mortality rate for bilateral lobectomy was 13 per cent in 1950.

There have been very few follow-up studies to determine the ultimate fate of patients treated by surgical excision; but, according to the information available, the persistence of symptoms after operation is not uncommon. Late complications may also occur which will be revealed only by the passage of time. For example, Lambert (25), in following 78 cases treated by excision, found that 10 (12.8 per cent) had developed active pulmonary tuberculosis within three years of operation and that three had died of this disease. This complication of lobectomy or pneumonectomy in the treatment of nontuberculous disease has been observed at this hospital, and 4 such cases have been reported by Katz (27).

It is believed that the present experience with bronchiectasis as a relatively benign disease will be broadly representative of what may be expected under similar circumstances elsewhere. The point to be made and emphasized is not

that the earlier studies were incorrect, but that a grave error will be made if all cases of bronchiectasis are treated on the basis of observations made under such intrinsically selective conditions as exist in a general hospital. Selective factors of an opposite nature are undoubtedly present in a clinic service. Acutely ill patients or patients with marked symptoms and dangerous complications will not be seen often in ambulant clinics, while relatively asymptomatic and uncomplicated bronchiectasis will be discovered frequently if bronchography is used freely as a diagnostic aid.

All cases of bronchiectasis encountered in a clinic service will not be benign in type, however, and all patients seen on a hospital ward will not present the disease in its malignant form. As with pulmonary tuberculosis, treatment of this disease cannot be a routine medical or surgical procedure but must vary, depending on the type and extent of the ectasia and in accordance with the clinican's interpretation of the preceding history and the presenting symptoms and signs. A number of other factors, including age, sex, occupation, and social status, as well as the presence or absence of other disease, will often influence a decision in regard to treatment. If a poor prognosis is given merely because bronchographic study has revealed bronchial ectasia which cannot be treated surgically, many patients and their families will be subjected to needless apprehension and many physicians to future embarrassment. Patients must be selected carefully for surgical treatment, and surgery should be avoided when treating benign forms of the disease if the operative and postoperative risks are not minimal. In short, as with most other chronic diseases, when a diagnosis of bronchiectasis is made, the first requirement is an adequate period of observation and evaluation and not immediate radical treatment.

Summary

A follow-up study is reported in which 49 ambulant clinic patients were observed for periods of 9 to 20 years after bronchiectasis was discovered by bronchographic examination. The mean follow-up period was 12.5 years.

Eight of the patients died during the follow-up period, but 3 deaths were not attributable to bronchiectasis.

In 41 surviving patients, there was clinical evidence of progression in only 3 instances. More than half of the group experienced an amelioration of symptoms.

Physical incapacity, due to any cause except aging, was absent in 75 per cent of the surviving patients. The complications usually expected in association with bronchiectasis were relatively rare.

Evidence of pulmonary disease by physical examination and routine roentgenography was commonly present at the time of diagnosis and, as a rule, such abnormalities persisted despite the absence of significant symptoms or complications.

In retrospect, there appears to have been an appreciable clinical difference between the fatal and the surviving groups at the time of diagnosis.

The prognosis in bronchiectasis in respect to groups of patients may vary widely under differing circumstances. Bronchiectasis as encountered in military hospitals or ambulant chest clinics tends to follow a much more benign course

than it does in patients seen on the wards of a large general hospital. In most textbooks and medical journals, the opinions expressed in regard to prognosis and treatment are based exclusively on studies carried out in large general hospitals. Applied to medical practice outside such hospitals, these opinions often prove to be unduly pessimistic and misleading.

It is concluded that the prognosis, and consequently the most desirable treatment, for any individual patient can be decided only on the basis of thorough clinical study, often requiring a period of judicious observation. Treatment by pulmonary resection is of unquestioned value in many cases, but surgery is not indicated whenever resectable bronchiectasis is discovered.

Sumario

La Bronquiectasia según se Observó en un Servicio Clínico Ambulante: Estudio de 49 Casos Seguidos Durante un Período Mínimo de Nueve Años

En este estudio continuado, 49 efermos ambulantes de una clínica fueron mantenidos en observación durante períodos de tiempo de 9 a 20 años después de descubrirse la bronquiectasia con el examen broncográfico. El período medio de observación fué de 12.5 años.

Ocho de los pacientes fallecieron durante el período de observación, pero 3 de las muertes no son imputables a la bronquiectasia.

De 41 enfermos sobrevivientes, sólo en 3 hubo signos clínicos de agravación. Más de la mitad del grupo experimentó mejoría de los síntomas.

En 75 por ciento de los sobrevivientes, no hubo incapacidad física debida a causa alguna, aparte de la edad. Las complicaciones que suelen esperarse en asociación con la bronquiectasia fueron relativamente raras.

Al hacer el diagnóstico, había habitualmente signos de afección pulmonar revelados por el examen físico y la radiografía corriente, y por regla general, esas anomalías persistieron a pesar de la falta de síntomas o complicaciones de importancia.

Retrospectivamente, parece haber habido una diferencia clínica apreciable entre los grupos de fallecidos y de sobrevivientes al hacer el diagnóstico.

Ofrécese un repaso de la literatura médica pertinente y una reseña de la variable naturaleza de la bronquiectasia, con referencia particular a su frecuencia como enfermedad benigna o autolimitada.

Resume

La bronchiectasie, telle qu'elle est observée dans les dispensaires. Etude de l'observation de 49 cas suivis pendant une période minimum de neuf ans

Rapport fondé sur l'observation de malades ambulants pendant des périodes de 9 à 20 ans après que la bronchiectasie ait été découverte par la bronchographie. La période moyenne d'observation était de 12 ans ½.

Huit de ces malades sont morts au cours de la période d'observation, mais 3 de ces décès n'étaient pas imputables à la bronchiectasie.

Chez 41 des malades survivants, des signes d'évolution n'ont été observés que dans 3 cas. Une amélioration des symptômes s'est manifestée dans plus de la moitié des cas étudiés.

L'incapacité physique, déterminée par toute autre cause que l'âge, a été absente chez 75% des malades survivants. Les complications habituellement associées à la bronchiectasie ont été relativement rares.

Il existait des signes évidents d'une affection du poumon au moment où le diagnostic était fait par l'examen physique et par la radiographie périodique et, le plus souvent, ces altérations subsistaient en dépit de l'absence de symptômes significatifs ou de complications.

Rétrospectivement, une différence appréciable du point de vue clinique, paraît avoir existé, au moment du diagnostic, entre le groupe des décès et le groupe de survie.

Les publications médicales pertinentes sont passées en revue et le caractère variable de la bronchiectasie est étudié. Les auteurs insistent sur la fréquence de son apparition sous une forme bénigne ou circonscrite.

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ISONIAZID IN EXPERIMENTAL TUBERCULOSIS OF GUINEA PIGS INFECTED WITH TUBERCLE BACILLI RESISTANT TO STREPTOMYCIN AND TO PARA-AMINOSALICYLIC ACID[1]

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(Received for publication June 20, 1952)

It has been found that strains of *M. tuberculosis* resistant to streptomycin and to para-aminosalicylic acid (PAS) are as susceptible to the action of isoniazid (isonicotinic acid hydrazide) *in vitro* as are nonresistant strains (1–3). Furthermore, it has been reported that administration of isoniazid is effective against experimental tuberculosis in guinea pigs infected with streptomycin-resistant tubercle bacilli (2). The administration of isoniazid to patients from whom streptomycin-resistant tubercle bacilli were isolated was reported to produce a favorable therapeutic response (4).[2]

In the present study it was found that isoniazid produced a pronounced therapeutic effect in tuberculosis of guinea pigs infected with a strain of human-type tubercle bacillus which was resistant to streptomycin and to PAS.

METHODS

Each of 28 mature male guinea pigs which weighed approximately 600 gm. apiece was inoculated subcutaneously over the sternum with 0.1 mg. (moist weight) of a human type of tubercle bacillus which was resistant to more than 1,000γ of streptomycin and to more than 64γ of PAS per ml. of egg-yolk agar medium.[3] On the fourteenth day of infection, 2 animals were killed, one of which had grossly visible tuberculous lesions in the spleen; the other had similar lesions in the spleen and liver. Both had tuberculous lesions at the site of inoculation and in the axillary lymph nodes. On the twentieth day of infection, 2 additional animals were killed. Lesions of tuberculosis were present in the lungs, liver, spleen, site of inoculation, and the axillary lymph nodes (pretreatment controls, figure 1). It was assumed that each of the 24 remaining animals had lesions of tuberculosis comparable in extent to those seen in the pretreatment control animals.

On the twentieth day of infection, the remaining 24 animals were divided into four groups of 6 each, as shown in figure 1. One group served as untreated control animals. Treatment with streptomycin and PAS, respectively, was begun in the second and third groups to learn whether or not the infecting microorganism was resistant to these drugs *in vivo*. The streptomycin was given to each animal in a single daily dose of 6 mg. in 1 ml. of solution injected into the axillary space. The PAS was given once daily by means of a gastric tube in the form of 100 mg. of the sodium salt (NaPAS) in 1 ml. of solution. These amounts of streptomycin and of NaPAS will cause regression and healing of experimental tuberculosis in guinea pigs infected with 0.1 mg. of drug-sensitive tubercle

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[1] From the Division of Experimental Medicine, Mayo Foundation, University of Minnesota, Rochester, Minnesota.

[2] For the first reports on the experimental and clinical use of isoniazid, the reader is referred to the following: *The Quarterly Bulletin of Sea View Hospital* (5), January, 1952; *Diseases of the Chest* (6), April, 1952; *The American Review of Tuberculosis* (7), April, 1952; and to the subsequent review in the *Lancet* (8) and to the report by Fust (9).

[3] This organism was isolated from a patient who was treated first with streptomycin and then with PAS.

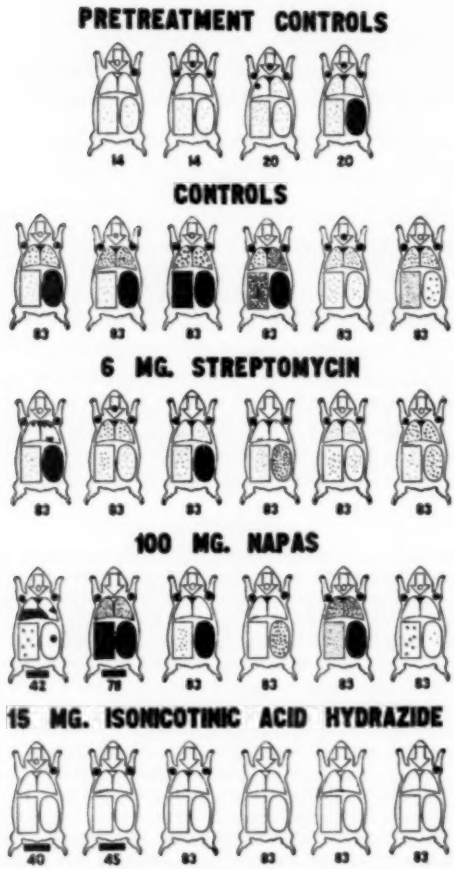

FIG. 1. Schematic representation of the extent of disease observed in each animal at necropsy after sixty-three days of treatment, which was begun twenty days after infection. The rectangle represents the liver and the oval represents the spleen. Small and large dots indicate miliary and nodular lesions, respectively. Complete blackening indicates severe diffuse involvement of the organ. A circle or a dot in the arrow represents an ulcer or an abscess, respectively, at the site of inoculation. A large dot in the axillary space indicates involvement of the regional lymph nodes. The numerals indicate the number of days of infection. A black bar means that the animal died. No visible lesions were present in the lungs, liver, or spleen of the animals treated with isoniazid, which is in contrast to the extensive disease encountered in the other animals.

bacilli if treatment is continued for sixty days, starting twenty days after infection. The dose of isoniazid[4] was selected by determining the amount of a single daily oral dose that could be tolerated without loss of weight when the drug was administered in 1 ml. of solu-

[4] The isoniazid was furnished by E. R. Squibb & Sons, New Brunswick, New Jersey.

tion by means of a gastric tube. When the drug was given at the rate of 10 mg. per day for three days, followed by 15 mg. daily for three more days, an average gain in body weight of 10 to 20 gm. per animal was noted. When the daily dose was increased to 25 mg., the animals continued to gain in weight for six days but, by the eighth day, 3 of the 6 animals had lost approximately 10 gm. each, while the other 3 had gained only slightly. On the ninth day of treatment, the dose was reduced to 15 mg. once daily per animal and maintained at that level for the remaining forty-nine days of treatment.[5]

After sixty-three days of treatment, which began on the twentieth day of infection, all surviving animals were killed. A record was made of the extent of tuberculous disease seen at necropsy. Portions of lung, liver, kidney, spleen, site of inoculation, and axillary lymph nodes were preserved for histopathologic study. A portion of spleen taken aseptically from the untreated controls and from the animals treated with isoniazid was cultured for tubercle bacilli.

RESULTS

Mortality: Of the 6 animals treated with isoniazid, one died on the twentieth day of treatment. No visible lesions of tuberculosis occurred in the internal organs, but an ulcer was noted at the site of inoculation and an enlarged axillary lymph node was present. The cause of death could not be determined. Another guinea pig in this group died on the twenty-fifth day of treatment, two days after having bitten off and swallowed approximately 8 cm. of the ureteral catheter being used for intubation. Necropsy of this animal disclosed purulent mediastinitis and pleuritis, but no demonstrable tuberculous lesions were present in the internal organs. However, enlargement of the axillary lymph nodes was noted. The liver was diffusely yellowish in color in both these animals.

Two animals treated with NaPAS died on the twenty-second and fifty-eighth days of treatment. The cause of death was not determined for the first of these animals but, in the latter, sufficient tuberculous involvement of the lungs, liver, and spleen was present to have been the cause of death.

Changes in weight: All animals living at the end of the study had gained weight. The untreated control animals, those given streptomycin, and those treated with NaPAS each gained an average of 120 gm. The surviving animals treated with isoniazid had gained an average of 220 gm., which indicated that the amount of drug given daily was well tolerated.

Observations at necropsy: Extensive tuberculous involvement of the usual organs of predilection occurred in the untreated control animals as well as in those animals treated with streptomycin or with NaPAS (figure 1). In great contrast was the absence of any grossly visible disease in the lungs, liver, and spleen of the animals treated with isoniazid. Only the animal which died after twenty days of treatment had a residual visible lesion at the site of inoculation. Four animals had involvement of the axillary lymph nodes, but in the remaining 2 animals these lymph nodes were apparently normal.

[5] In a group of 4 normal guinea pigs given the same treatment with isoniazid as herein described, 3 lost weight and one gained slightly after receiving 25 mg. once daily for three days. The daily dose was then increased to 50 mg., which produced gradual loss of weight until the animals died five, eight, nine, and eleven days, respectively, after the onset of the higher daily dose. At necropsy, each animal displayed pronounced yellowish discoloration of the liver.

Histopathology: The microscopic appearance of the lesions in the untreated animals and in those treated with streptomycin or NaPAS was characteristic of the widespread and necrotic tuberculous process seen in experimentally infected guinea pigs (figure 2*a*). In the 2 pretreatment control animals killed after fourteen days of infection, small but progressive lesions were present in the liver and spleen; whereas those killed at twenty days had multiple small active

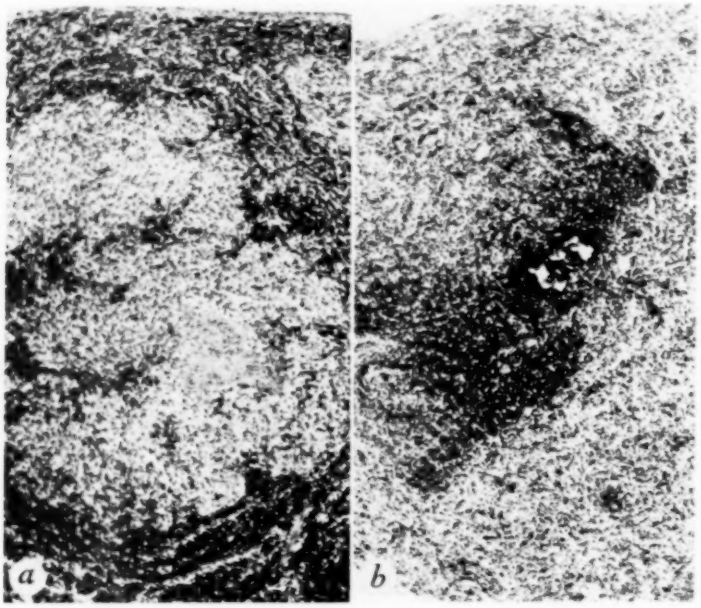

FIG. 2*a*. Progressive necrotic lesions of the spleen of an untreated animal killed after eighty-three days of infection. This was typical of the lesions in the internal organs of all of the controls as well as of those treated with streptomycin and those treated with PAS (hematoxylin and eosin; × 55). *b*. Small calcified focus in the spleen of an animal treated for sixty-three days with isoniazid, starting twenty days after infection (hematoxylin and eosin; × 55).

tubercles in the lungs in addition to conglomerate necrotic lesions in the liver and spleen. It is assumed that all animals had lesions comparable in extent and character on the twentieth day of infection, when treatment was begun. No active lesions of tuberculosis were seen in sections from the lungs, liver, or spleen of any animal treated with isoniazid, including the 2 animals which died after only twenty and twenty-five days of treatment. Five animals, however, had microscopically active tuberculous foci in the axillary lymph nodes. In the liver of one animal and in one lung of another, a small fibrotic scar was present. A calcified region was noted in the spleen of a third animal (figure 2*b*). Other than

these lesions, no microscopic evidence was found of the disease which is assumed to have been present when treatment with isoniazid was begun.

Diffuse changes resembling fatty metamorphosis were present in the livers of the animals which died on the twentieth and twenty-fifth days of treatment with isoniazid (figure 3). These changes were present to a limited degree in 3 of the other 4 animals that received isoniazid. One animal treated with 100 mg.

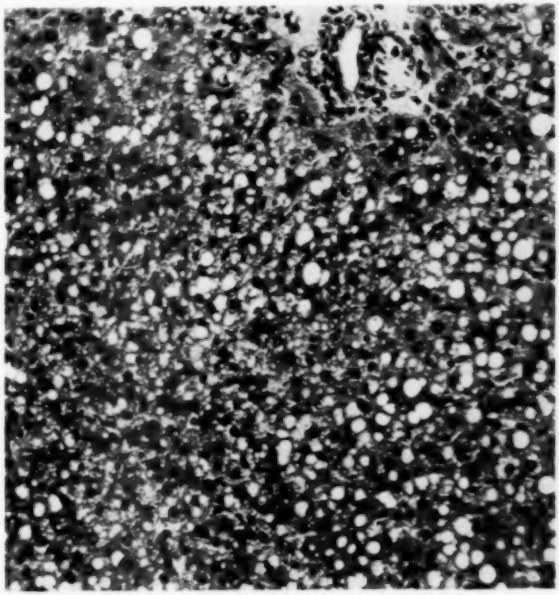

FIG. 3. Fatty metamorphosis in the liver of the guinea pig which died after twenty-five days of treatment with isoniazid. The drug had been given in doses of 10 mg. for three days, 15 mg. for three days, 25 mg. for eight days, and 15 mg. for eleven days (hematoxylin and eosin; × 180).

of NaPAS daily died from unknown causes after twenty-two days of treatment. Slight but diffuse fatty change was present in the liver, but this was not seen in any other animal.[6]

Bacteriologic examination: A positive culture was obtained from the axillary lymph node but not from the spleen of the animal which died after twenty days of treatment with isoniazid. The spleen of the animal which died on the

[6] Extensive diffuse vacuolation of the hepatic cells was seen in each of the 4 noninfected animals which died while being treated with 50 mg. of isoniazid daily. Innumerable tubular casts were present in the kidneys of each of these animals and a large amount of acidophilic material was noted around most of the glomeruli (figure 4). This was not seen in the infected animals, in which lower doses were used.

twenty-fifth day of treatment yielded a positive culture, but cultures of the spleens of the 4 animals which completed the treatment showed no growth after eighty days. Cultures of the spleens of each of the untreated animals were positive within thirty days.

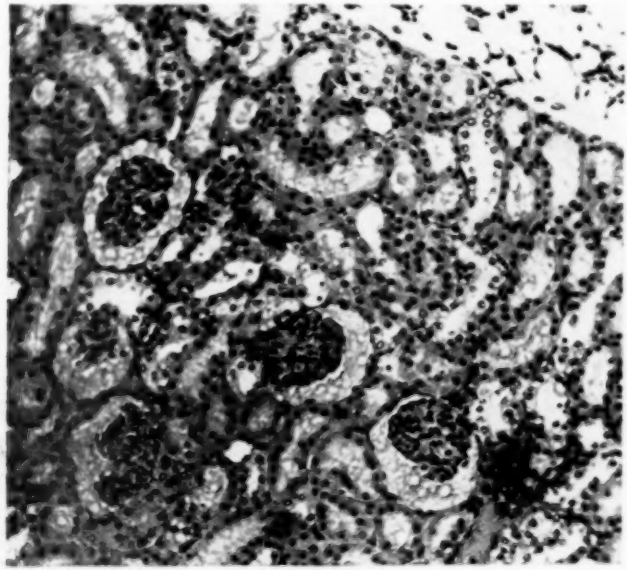

FIG. 4. Section of kidney from a noninfected guinea pig which died after being treated for eight days with isoniazid in single daily doses of 50 mg. (hematoxylin and eosin; × 140).

COMMENT

The extent of lesions observed in the pretreatment control animals, especially in those killed on the twentieth day of infection, indicates that a widespread progressing disease had been established before treatment was started. This is important because it presents a formidable test of the therapeutic, rather than the prophylactic, property of a new chemotherapeutic agent. The absence of active disease in the lungs, liver, and spleen of any of the animals treated with isoniazid shows that, not only inhibition of the disease took place, but also regression and healing of the morbid changes present when treatment was started.

The oral dose of 15 mg. of isoniazid given once daily for the last forty-nine days of treatment amounts to approximately 20 mg. per kg. of body weight. This is comparable to the subcutaneous dose of isoniazid that Uehlinger, Siebenmann, and Frei (10) found to be as effective in tuberculosis of guinea pigs as a combination of 20 mg. of streptomycin plus 1 gm. of PAS daily per kg. It is probable, therefore, that the optimal daily therapeutic dose of isoniazid for tuberculosis in guinea pigs is less than 20 mg. per kg. of body weight.

Uehlinger, Siebenmann, and Frei also reported that little evidence of toxicity of isoniazid was noted for guinea pigs at a daily dose of 20 mg. per kg. of body weight. Steenken and Wolinsky (*7b*) found that a daily oral dose of 40 mg. of isoniazid per kg. of body weight was tolerated by guinea pigs for two weeks. In the present experiment, some indication existed that single daily doses of 25 mg. per animal (33 mg. per kg.) were not well tolerated, since 3 of the 6 animals so treated lost weight after eight days of treatment with this dose. Two animals which died six and eleven days, respectively, after the daily dose had been reduced from 25 to 15 mg. showed extensive fatty change in the liver. Whether this is related to the medication is not apparent. However, extensive fatty metamorphosis was seen in each of 4 noninfected animals which died after receiving 50 mg. (66 mg. per kg.) once daily for four, eight, nine, and eleven days, respectively.

Rubin and associates (*7d*) found that chronic administration of isoniazid to dogs resulted in fatty degeneration of the liver, which appeared to be reversible when administration of the drug was stopped.

SUMMARY

Twenty-eight guinea pigs were infected with a strain of human-type tubercle bacillus resistant *in vitro* to streptomycin and to para-aminosalicylic acid (PAS). Two animals killed on the fourteenth day and 2 killed on the twentieth day of infection, before treatment was begun, were found to have visible lesions of tuberculosis. The remaining 24 animals were divided into four groups of 6 each as follows: (*1*) untreated controls; (*2*) those treated with 6 mg. of streptomycin once daily; (*3*) those treated with 100 mg. of the sodium salt of para-aminosalicylic acid by intubation once daily; and (*4*) those treated with isoniazid once daily by intubation in doses of 10 mg. for three days, 15 mg. for three days, 25 mg. for eight days, and 15 mg. for forty-nine days. The period of treatment started on the twentieth day of infection and continued for sixty-three days.

Two animals given isoniazid which died on the twentieth and twenty-fifth days of treatment, respectively, had no gross or microscopic evidence of active tuberculous lesions. At the end of the period of treatment all survivors were killed. Widespread destructive tuberculous disease was present in the untreated animals, as well as in those given streptomycin and those given the sodium salt of PAS. In marked contrast was the absence of active lesions, as determined by gross and microscopic examination of the lungs, liver, and spleen, in any of the animals given isoniazid.

It is concluded that, by the methods used in this study, isoniazid effects healing of progressive tuberculosis in guinea pigs caused by tubercle bacilli resistant to streptomycin and to PAS.

SUMARIO

La Hidracida del Acido Isonicotínico (Isoniacida) en la Tuberculosis Experimental de los Cobayos Infectados con Bacilos Tuberculosos Resistentes a la Estreptomicina y al Acido Para-Aminosalicílico (PAS)

Veintiocho cobayos fueron infectados con una cepa tipo humano del bacilo tuberculoso, resistente *in vitro* a la estreptomicina y al ácido para-aminosalicílico (PAS). En 2 animales

matados al décimocuarto día y 2 matados al vigésimo día de la infección, antes de iniciar el tratamiento, se encontraron lesiones visibles de tuberculosis. Los 24 animales restantes fueron divididos en cuatro grupos de a 6 cada uno en la forma siguiente: (*1*) testigos no tratados; (*2*) tratados con 6 mg. de estreptomicina una vez diaria; (*3*) tratados con 100 mg. de la sal sódica del ácido para-aminosalicílico por intubación una vez diaria; y (*4*) tratados con isoniacida una vez diaria por intubación a dosis de 10 mg. durante tres días, 15 mg. durante tres días, 25 mg. durante ocho días y 15 mg. durante cuarenta y nueve días. El período de tratamiento comenzó el vigésimo día de la infección y continuó por espacio de sesenta y tres días.

Dos animales que recibieron isoniacida y que murieron a los veinte y veinte y cinco días de tratamiento, no tenían signos macro- o microscópicos de lesiones tuberculosas activas. Al terminar el período de tratamiento, se mató a todos los sobrevivientes. Había presente enfermedad tuberculosa destructiva generalizada en los animales no tratados e igualmente en los que recibieron estreptomicina y los que recibieron la sal sódica del PAS. Formaba decidido contraste con esto la ausencia de lesiones activas, según determinó el examen macro- y microscópico de los pulmones, hígado y bazo, en todos los que recibieron isoniacida.

Dedúcese que, con los métodos usados en este estudio, la isoniacida efectúa la cicatrización de la tuberculosis evolutiva ocasionada en los cobayos por bacilos tuberculosos resistentes a la estreptomicina y al PAS.

RESUME

Hydrazide de l'acide nicotinique (Isoniazide) dans la tuberculose expérimentale du cobaye inoculé avec des bacilles tuberculeux streptomycino et P.A.S. résistants

Vingt-huit cobayes ont été inoculés avec le bacille tuberleux humain résistant *in vitro* à la streptomycine et à l'acide para-aminosalicylique (P.A.S.). Deux animaux, sacrifiés au quatorzième jour de l'infection, avant l'instauration du traitement, ont présenté des lésions tuberculeuses apparentes. Les autres 24 animaux ont été répartis, par groupe de 6, de la manière suivante: (*1*) témoins non traités; (*2*) animaux traités avec 6 mg. de streptomycine, une fois par jour; (*3*) animaux traités par intubation quotidienne avec le sel de sodium de l'acide para-aminosalicylique à la dose de 100 mg. par jour; (*4*) animaux traités par intubation quotidienne d'isoniazide à la dose de 10 mg. pendant trois jour, 25 mg. pendant huit jours et 15 mg. pendant quarante-neuf jours. La période de traitement commençait au vingtième jour de l'inoculation et se poursuivait pendant soixante-trois jours.

Deux des animaux ayant reçu l'izoniazide, et qui sont morts le vingtième et le vingt-cinquième jour du traitement, respectivement, ne présentaient aucun signe macroscopique ou microscopique de tuberculose en activité. A la fin de la période de traitement tous les survivants ont été sacrifiés. Des lésions tuberculeuses destructrices disséminées ont été observées aussi bien chez ceux des animaux traités par la streptomycine que chez ceux traités par le sel de sodium du P.A.S. Ceci était en contraste frappant avec l'absence de lésions actives, confirmée par l'examen macroscopique et microscopique des poumons, du foie et de la rate chez chacun des animaux recevant l'isoniazide.

La conclusion des auteurs est la suivante: dans les conditions expérimentale de cette étude, l'isoniazide provoque la cicatrisation des lésions dans la tuberculose du cobaye causée par des bacilles streptomycino et P.A.S. résistants.

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THE IN VIVO AND IN VITRO EFFECT OF STREPTOMYCIN ON STREPTOMYCIN-RESISTANT TUBERCLE BACILLI[1, 2]

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(Received for publication April 23, 1952)

INTRODUCTION

A knowledge of the effect of streptomycin on the course of tuberculous disease either caused or perpetuated by tubercle bacilli highly resistant to this drug is of great practical importance. In the past, various workers such as Youmans and Williston (1), Feldman, Karlson, and Hinshaw (2), Emmart, McClosky, Smith, and Lieberman (3), and Pratt and Steenken (4) have reported that animals experimentally infected with virulent mammalian tubercle bacilli resistant to streptomycin did not respond favorably to treatment with this drug. In human tuberculosis, streptomycin therapy is generally considered ineffective following the emergence to predominance of drug-resistant tubercle bacilli. Steenken and Wolinsky (5) reported that streptomycin treatment exerted a deleterious effect in experiments in which two groups of guinea pigs were infected with highly streptomycin-resistant tubercle bacilli by either the subcutaneous route or the inhalation of bacteria. In these experiments, streptomycin-treated animals began to succumb sooner and at a more rapid rate than the untreated control animals. These investigators do not believe, however, that this observation can be explained either on the basis of increased virulence of the tubercle bacilli or by their development of streptomycin dependence, since they failed in their attempts to demonstrate that either of the two phenomena actually had occurred. That treatment with streptomycin in infections caused by streptomycin-resistant variants of certain bacteria might enhance their virulence is suggested by the work of Miller and Bohnhoff (6) and Hall and Spink (7), who had previously reported that streptomycin treatment resulted in enhanced virulence of streptomycin-resistant strains of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and of *Brucella abortus*. The latter workers also noticed the stimulating influence of streptomycin on the growth of streptomycin-resistant strains of *B. abortus* in various culture media. McDermott, Muschenheim, Hadley, Bunn, and Gorman (8) and, later, Deyke, Fisher, James, and Sides (9) drew attention to the grave potentialities which might be inferred from these observations as far as continuation of treatment of human tuberculosis with streptomycin in the presence of drug-resistant organisms is concerned.

Quantitative data concerning the effect of streptomycin on the growth *in vitro* of streptomycin-resistant tubercle bacilli are as yet not available. The work of Steenken and Wolinsky (5) on the effect of streptomycin treatment on animals

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[2] This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.

experimentally infected with tubercle bacilli resistant to this drug was not sufficiently quantitative to warrant any conclusion concerning the possible deleterious effect of streptomycin treatment on the course of tuberculosis caused by streptomycin-resistant tubercle bacilli. It was thought desirable, therefore, to conduct experiments with sufficiently quantitative methods in order to determine the effect of streptomycin on experimental tuberculosis caused by streptomycin-resistant tubercle bacilli and on the growth *in vitro* of such organisms.

MATERIALS AND METHODS

In Vivo Experiments

Albino mice of the "Strong A" strain were employed in the *in vivo* experiments. In the first series of experiments the mice, weighing approximately 20 gm., were divided into three groups; each one consisted of approximately 40 animals. All mice were infected by the intravenous inoculation of 0.75 mg. of tubercle bacilli suspended in 0.4 ml. of 0.01 molar phosphate buffer, pH 7.0. The tubercle bacilli employed in the experiment were isolated from the sputum of humans with pulmonary tuberculosis who had been under streptomycin treatment for various lengths of time.

The strains of *Mycobacterium tuberculosis* were designated as *M. tuberculosis* "3609," *M. tuberculosis* "3551," and *M. tuberculosis* "2974," respectively. On egg-yolk agar medium containing various concentrations of streptomycin, these organisms were found to be fully resistant to streptomycin in concentrations of 1,000 γ per ml. of medium. Prior to their use in the experiments, the organisms were grown for fourteen days on slants of Herrold's egg-yolk medium containing streptomycin in a concentration of 1,000 γ per ml. Suspensions for the inoculation of the mice were prepared by grinding the growth from these cultures with buffer solution in a porcelain mortar. The concentration of bacteria in the suspensions was determined by centrifugation in Hopkins vaccine tubes and, if necessary, adjusted by diluting with buffer solution.

Each of the three groups of mice was injected into the lateral tail vein with one of the three respective strains of tubercle bacilli.

Streptomycin treatment of half of the animals in each group was started the day following their infection. These animals were injected daily with 3,000 γ of streptomycin contained in a total volume of 0.2 ml. of distilled water. The injections were made under the skin at the nape of the neck with tuberculin syringes equipped with 27-gauge hypodermic needles. The remaining animals in each group served as controls. They were injected daily with 0.2 ml. of distilled water in a manner identical with the one described for the streptomycin-treated animals. All injections were continued until death of the animals.

An additional series of *in vivo* experiments was carried out in two groups of mice, each consisting of approximately 80 animals. The mice in these experiments were somewhat older than the animals in the previous series and had an averge weight of 30 gm. All animals in this series were infected by the intravenous injection of 1 mg. of tubercle bacilli contained in a total volume of 0.4 ml. The method of growing the bacteria and of preparing the bacterial suspensions for inoculation was identical with the one described above. The tubercle bacilli employed in this series of experiments were designated as *M. tuberculosis* "2515" and *M. tuberculosis* "3764," respectively. They were obtained from the sputum of tuberculous patients under treatment with streptomycin. Both of these strains were found to be fully resistant to a streptomycin concentration of 5,000 γ per ml. on egg-yolk agar slants. Each of the two groups of mice was injected into the lateral tail vein with one of the two strains of *M. tuberculosis*. Each group was then divided into four numerically equal subgroups.

One of the subgroups was treated with streptomycin in a manner identical with the one described above. The second subgroup of animals received injections with an equal amount

of streptomycin which had been inactivated, however, by autoclaving for thirty minutes at a temperature of 121°C. The third subgroup of mice received daily injections of distilled water. The remaining group of animals did not receive any treatment. The deaths which occurred in each of the groups of mice were recorded daily. Autopsies were performed in order to exclude the possibility that death might have been due to causes other than tuberculosis. The cumulative percentage dead in each subgroup of animals was calculated and plotted on logarithmic probability paper against time. The median survival times and their 95 per cent confidence limits were calculated from the mortality data according to the method of Litchfield (10).

In Vitro Experiments

Two strains of *M. tuberculosis* were used in the *in vitro* experiments on the effect of streptomycin on the growth of streptomycin-resistant tubercle bacilli. One of the strains was *M. tuberculosis* "3764," whose derivation and pertinent characteristics have been described above. The other strain was *Mycobacterium tuberculosis* H37RvR, a streptomycin-resistant variant of the Trudeau strain of *M. tuberculosis* H37Rv, which had been produced in this laboratory several years ago by Williston and Youmans (11). On Herrold's egg-yolk slants, this organism was found to be completely resistant to streptomycin in concentrations of 1,000 γ per ml., but it failed to grow in the presence of 5,000 γ of streptomycin per ml. of medium.

The culture medium employed was a synthetic basal medium of the following composition, to which was added bovine albumin and Tween 80[3]: asparagine, 0.5 per cent; monopotassium phosphate, 0.5 per cent; potassium sulfate, 0.05 per cent; magnesium citrate, 0.15 per cent; and Tween 80, 0.02 per cent. Prior to autoclaving, the pH was adjusted to 6.8 to 7.0; following sterilization, bovine albumin (fraction V, Armour) and glucose were added to give a final concentration of 0.2 and 0.5 per cent, respectively. The medium was then dispensed in 7.0 ml. amounts in aluminum-capped round optical cuvettes (Coleman "7-304") measuring 19 by 150 mm. To some of these tubes was added aseptically 0.1 ml. of distilled water, while other tubes received 0.1 ml. volumes of distilled water containing appropriate concentrations of streptomycin to bring the final streptomycin concentrations in these tubes to 10, 100, and 1,000 γ per ml. of medium, respectively. The inocula used consisted of 0.1 ml. volumes from eight-day-old cultures of the two organisms in the above medium. Ten tubes of each of the three streptomycin concentrations and ten tubes of medium not containing any streptomycin were inoculated respectively with each of the two strains. The tubes were incubated in an upright position in an air incubator at 37 ± 1° C., and growth was measured daily nephelometrically with the Coleman Model 7 photonephelometer for a total of fifteen days. Prior to each daily determination, each tube was well shaken so that smoothly turbid suspensions were obtained.

This method makes it possible to follow the growth of *M. tuberculosis* through its various growth phases, including the phase of "arithmetic linear" growth, which has been recently described by Fisher, Kirchheimer, and Hess (12) to occur regularly with this organism under the conditions of the present experiment.

Graphs representing the growth of the organisms were prepared by plotting the optical density measurements in "nephelos" units against time on a semi-logarithmic, as well as on an arithmetic, scale. With the photonephelometer employed here, "nephelos" units correspond directly to the bacterial concentration, and in this medium one such unit was found to be equivalent to approximately 500,000 tubercle bacilli per ml. The generation times (GT) of the organisms growing in

[3] Proprietary name of the polyoxyethylene derivative of Sorbitan monooleate, obtained from the Atlas Powder Co., Wilmington, Delaware.

each one of the cuvettes were calculated from their respective logarithmic growth rates according to the equation: $GT = \frac{\log 2}{r}$ where r represents the logarithmic rate of growth. The arithmetic growth rates (r_a) were calculated from numerical density values during the arithmetic linear part of the curve by use of the equation $r_a = \frac{N_2 - N_1}{t}$. N_2 is the optical density in "nephelos" units at the end of time interval t. N_1 is the optical density in "nephelos" units at the beginning of time interval t. The statistical significance of the differences in generation time and arithmetic growth rates, respectively, between organisms growing in medium not containing any streptomycin and in the same medium containing various concentrations of the drug were determined by calculating the probable errors of the differences of means and the respective t values.

RESULTS

The results of the various *in vivo* experiments are summarized in tables 1, 2, and 3. The statistical analysis shows that in all instances except in the experiment with *M. tuberculosis* "2974" and with *M. tuberculosis* "3609" there was no significant difference in the median survival times of the mice treated with streptomycin and those which had received instead injections with either heat-inactivated streptomycin or distilled water, or received no treatment at all. As may be seen from the results summarized in table 1, the streptomycin-treated animals which had been infected with *M. tuberculosis* "2974" and with *M. tuberculosis* "3609" had a significantly longer median survival time than the control animals which had received injections of distilled water.

Figure 1 is a plot of the means of the arithmetic and logarithmic values, respectively, of the optical densities in "nephelos" units of *M. tuberculosis* H37RvR growing in the medium not containing any streptomycin. This graph is presented here in order to illustrate the type of growth curves obtained in these experiments. In table 4 are presented the means of the generation times and the means of the arithmetic rates together with their respective probable errors.

The results of these growth experiments show that streptomycin in concentrations of 1,000 γ per ml. of medium significantly prolonged the generation time of both organisms.

Lesser concentrations of streptomycin did not significantly change the generation times of either of the two strains of *M. tuberculosis*. They had a significant effect, however, on the arithmetic growth rates. With *M. tuberculosis* "3764," the presence of 100 γ and 1,000 γ of streptomycin significantly depressed the arithmetic growth rate. The decrease of the arithmetic growth rate of this organism in the presence of 10 γ of streptomycin is probably not statistically significant. The arithmetic growth rates of *M. tuberculosis* H37RvR were significantly reduced in the presence of 10 γ and of 100 γ of streptomycin per ml. of medium. Peculiarly, the arithmetic rate of growth of *M. tuberculosis* H37RvR in the presence of 1,000 γ of streptomycin was slightly greater than the one in the medium not containing any streptomycin. With *M. tuberculosis* "3764," the arithmetic

TABLE 1

THE EFFECT OF STREPTOMYCIN TREATMENT ON THE SURVIVAL OF MICE INFECTED WITH THE STREPTOMYCIN-RESISTANT STRAINS "3609", "3551", AND "2974" OF M. TUBERCULOSIS VAR. HOMINIS

INFECTED WITH M. TUBERCULOSIS "3609"				INFECTED WITH M. TUBERCULOSIS "3551"				INFECTED WITH M. TUBERCULOSIS "2974"			
Treated with Streptomycin		Treated with Distilled Water		Treated with Streptomycin		Treated with Distilled Water		Treated with Streptomycin		Treated with Distilled Water	
Survival Ratio*	Days after Infection	Survival Ratio	Days after Infection	Survival Ratio	Days after Infection	Survival Ratio	Days after Infection	Survival Ratio	Days after Infection	Survival Ratio	Days after Infection
19/20	20	17/18	17	17/18	12	19/20	11	18/20	26	17/18	9
16/20	22	16/18	18	16/18	13	17/20	12	17/20	29	16/18	18
15/20	23	15/18	19	12/18	14	12/20	13	16/20	31	14/18	21
14/20	24	14/18	20	10/18	17	9/20	14	15/20	33	13/18	22
13/20	25	12/18	21	9/18	18	6/20	15	14/20	36	12/18	23
11/20	26	11/18	22	8/18	19	3/20	16	13/20	46	11/18	25
10/20	28	4/18	23	6/18	26	2/20	17	12/20	49	10/18	27
9/20	33	3/18	24	5/18	30	1/20	18	11/20	51	9/18	28
8/20	34	1/18	26	4/18	38	0/20	19	10/20	52	7/18	29
7/20	36	0/18	39	3/18	40			9/20	53	6/18	32
6/20	38			2/18	41			8/20	56	5/18	36
5/20	39			1/18	47			7/20	61	4/18	37
4/20	51			0/18	55			5/20	62	3/18	40
3/20	55							4/20	70	2/18	41
2/20	62							3/20	72	1/18	47
1/20	78							2/20	78	0/18	54
0/20	82							1/20	84		
								0/20	92		
Median Survival Times (95 Per Cent Confidence Limits)											
30 (24.0–37.5)		21.5 (20.5–22.5)		17.0 (11.7–24.6)		14.0 (13.2–14.7)		48.0 (39.3–58.5)		28.0 (23.7–33.0)	

* Survival ratio = $\frac{\text{number of survivors}}{\text{total number of mice}}$

TABLE 2

THE EFFECT OF STREPTOMYCIN TREATMENT ON THE SURVIVAL OF MICE INFECTED WITH THE STREPTOMYCIN-RESISTANT M. TUBERCULOSIS "3764"

TREATED WITH STREPTOMYCIN		TREATED WITH HEAT-INACTIVATED STREPTOMYCIN		TREATED WITH DISTILLED WATER		NOT TREATED	
Survival Ratio	Days after Infection	Survival Ratio	Days after Infection	Survival Ratio	Days after Infection	Survival Ratio	Days after Infection
15/17	11	16/21	12	18/19	11	19/20	12
11/17	12	13/21	13	17/19	12	15/20	13
5/17	13	11/21	14	11/19	13	14/20	14
4/17	14	5/21	15	10/19	14	12/20	15
1/17	15	2/21	16	6/19	15	7/20	16
0/17	45	1/21	17	4/19	16	6/20	17
				3/19	17	5/20	18
				2/19	18	4/20	19
				1/19	21	3/20	23
				0/19	32	2/20	24
						1/20	26
Median Survival Times (95 Per Cent Confidence Limit)							
12.5 (11.8–13.2)		13.0 (12.3–13.8)		13.5 (11.9–15.2)		15.0 (12.9–17.4)	

TABLE 3

THE EFFECT OF STREPTOMYCIN TREATMENT ON THE SURVIVAL OF MICE INFECTED WITH THE STREPTOMYCIN-RESISTANT M. TUBERCULOSIS "2515"

TREATED WITH STREPTOMYCIN		TREATED WITH HEAT-INACTIVATED STREPTOMYCIN		TREATED WITH DISTILLED WATER		NOT TREATED	
Survival Ratio	Days after Infection	Survival Ratio	Days after Infection	Survival Ratio	Days after Infection	Survival Ratio	Days after Infection
15/18	13	19/20	12	19/20	12	18/19	11
12/18	14	18/20	14	17/20	13	16/19	13
10/18	16	17/20	15	14/20	14	15/19	14
9/18	17	12/20	16	11/20	15	14/19	15
6/18	21	9/20	17	8/20	16	11/19	16
5/18	23	7/20	20	6/20	17	10/19	17
4/18	25	6/20	23	4/20	18	7/19	18
3/18	26	5/20	32	3/20	32	6/19	19
2/18	40	4/20	35	2/20	41	5/19	20
1/18	41	3/20	40	1/20	43	4/19	22
0/18	44	2/20	42			3/19	26
		1/20	44			2/19	34
Median Survival Time (95 Per Cent Confidence Limit)							
17.0 (14.2–20.2)		17.0 (12.0–23.4)		15.5 (14.6–16.4)		17.0 (15.0–19.2)	

rate was significantly depressed by streptomycin in a concentration of 1,000 γ per ml. This effect was less striking, however, than with the lesser concentrations of streptomycin.

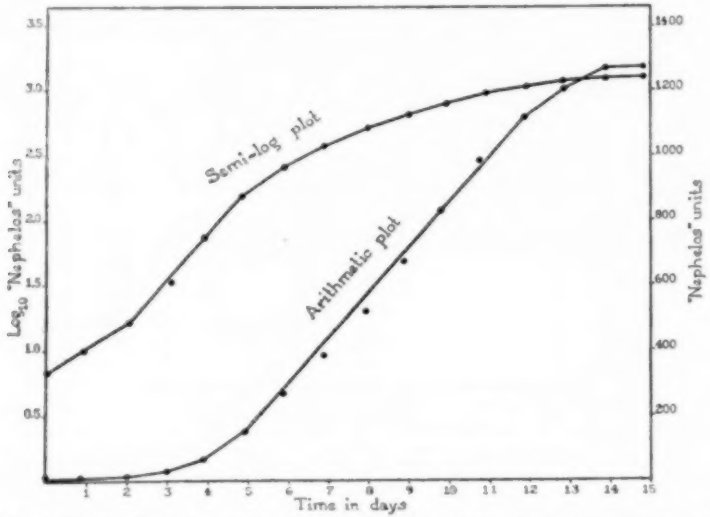

FIG. 1. Arithmetic and semi-logarithmic plots of nephelometric measurements of the growth of *Mycobacterium tuberculosis* var. *hominis* H37RvR in a streptomycin-free medium.

TABLE 4

GENERATION TIMES AND ARITHMETIC RATES OF GROWTH OF M. TUBERCULOSIS H37RvR AND M. TUBERCULOSIS "3764" IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF STREPTOMYCIN

	M. TUBERCULOSIS H37RvR				M. TUBERCULOSIS "3764"			
Concentration of streptomycin in γ per ml.....	0	10	100	1,000	0	10	100	1,000
Mean generation time in days ± probable error...	21.1 ±0.2	20.9 ±0.4	21.0 ±0.2	25.9 ±0.08	19.7 ±0.2	19.5 ±0.3	20.2 ±0.4	23.8 ±0.5
Mean arithmetic rate of growth ± probable error.....	5.73 ±0.07	5.30 ±0.05	5.13 ±0.04	5.92 ±0.06	5.58 ±0.14	5.27 ±0.08	4.85 ±0.13	5.04 ±0.08

DISCUSSION

The results of the *in vivo* experiments presented here show that streptomycin treatment of mice infected with tubercle bacilli highly resistant to this drug did not adversely affect their survival. In all but two instances, there was no

significant difference in the median survival time of the treated animals and the control animals. In the experiments with the mice which had been infected with *M. tuberculosis* "2974" and with *M. tuberculosis* "3609," respectively, streptomycin treatment actually seemed to benefit the animals, in spite of the fact that these strains of *M. tuberculosis* were fully resistant to 1,000 γ of streptomycin per ml. if tested on Herrold's egg-yolk medium. It should be pointed out, however, that the control animals in these groups which had received daily subcutaneous injections of distilled water seemed to be damaged by this treatment. Following prolonged treatment, they developed infiltrations and subcutaneous abscesses at the site of inoculation and this may possibly have contributed to their more rapid rate of death. In the remaining experiments, this effect of the injections with distilled water was either entirely absent or not equally pronounced, probably because these animals succumbed too soon for such damage to develop.

The method employed in the present *in vivo* experiments has the advantage of being more quantitative than the methods used previously in order to study the effect of streptomycin treatment on animals infected with tubercle bacilli resistant to this drug. The present method is similar to that reported by Baker, Schlosser, and White (13) and is based on the calculation of the median survival time and a statistical analysis of the data. It has been used recently by Youmans and Youmans (14) in order to evaluate the degree of antituberculous chemotherapeutic activity of various compounds. These workers also made use of this method in their work on the relation between the size of the infecting dose of tubercle bacilli and the survival time of mice (15).

The results of the experiments on the *in vitro* growth of streptomycin-resistant tubercle bacilli in the presence of streptomycin show that this drug does not enhance the growth of these organisms. On the contrary, the results indicate that concentrations of streptomycin to which the organism is fully resistant if tested on Herrold's egg-yolk medium significantly reduce the logarithmic and the arithmetic rate of growth. The determination of streptomycin resistance on slanted solid medium is not a quantitative procedure, however, and the differences in growth observed in the present experiments probably would escape detection by this method. On the other hand, the method employed here for the measurement of growth of tubercle bacilli makes use of liquid medium containing Tween 80 as a dispersing agent, and it should be pointed out that Fisher (16) has reported on the enhancing effect of Tween 80 on the streptomycin sensitivity of *Mycobacteria*. Significant decreases of the arithmetic rates of growth occurred in the presence of concentrations of streptomycin which did not significantly alter the growth of the organisms during their logarithmic phase of growth.

The logarithmic rates of growth were significantly reduced only in the presence of streptomycin in concentrations of 1,000 γ per ml. of medium, the highest concentration of drug used in these experiments. Peculiarly, this amount of streptomycin slightly enhanced the arithmetic rate of growth of *M. tuberculosis* H37RvR, whereas it depressed the arithmetic rate of growth of *M. tuberculosis* "3764." The arithmetic rates of growth of both organisms were significantly reduced by the lesser concentrations of drug employed in this study.

With *M. tuberculosis* H37RvR, it was further noticed that the duration of the lag phase was prolonged in the presence of 1,000 γ of streptomycin per ml. of medium. With the lower concentrations of streptomycin and with the control cultures not containing any streptomycin, the end of the lag phase was reached usually after two to three days. In the presence of 1,000 γ of streptomycin per ml. of medium, however, the end of the lag phase did not occur until after three to five days of incubation. Such an increased lag phase was not observed with *M. tuberculosis* "3764." It should be pointed out that Fisher, Kirchheimer, and Hess (12) have recently reported that a relatively long arithmetic linear growth phase occurs regularly with *M. tuberculosis* growing under the conditions of the present experiment. Furthermore, arithmetic linear growth seems to be a characteristic part of the cultural cycle of all members of the genus *Mycobacterium* (17) growing in liquid media in a dispersed state. There is evidence in addition to that presented here that the arithmetic rate of growth is a sensitive indicator of various environmental changes (18). This and the relatively prolonged duration of this growth phase suggest its use for quantitative studies on the effect of various agents and environmental changes on the growth of *Mycobacteria*.

In addition, it should be pointed out that, in general, the maximum population reached by the bacteria was progressively depressed in the presence of increasing concentrations of streptomycin. *M. tuberculosis* H37RvR growing in the presence of 1,000 γ of streptomycin, however, reached a higher maximum population than the same organism growing in lesser amounts of the drug. Nevertheless, the population thus attained was not significantly different from the maximum bacterial population in the tubes which contained no streptomycin.

SUMMARY

Mice were infected with five different streptomycin-resistant strains of tubercle bacilli and subsequently were treated with this drug. The results of these experiments show that streptomycin treatment did not exert a deleterious effect. In three of the five experiments, the median survival times of the streptomycin-treated animals did not significantly differ from the median survival times of control animals. In the remaining two experiments, the median survival times of the streptomycin-treated mice were significantly greater than those of control animals treated with distilled water. These differences in median survival times might have been the result of the damaging effects of prolonged treatment with distilled water.

Quantitative growth studies *in vitro* showed that the growth of two strains of streptomycin-resistant tubercle bacilli was not enhanced by the presence of this drug. Streptomycin in concentrations of 10 and 100 γ per ml. of medium significantly decreased the arithmetic rate of growth but did not have a significant effect on the logarithmic growth phase. The rate of logarithmic growth was significantly decreased, however, by streptomycin in concentrations of 1,000 γ per ml. of medium.

SUMARIO

El Efecto in Vivo e in Vitro de la Estreptomicina sobre los Bacilos Tuberculosos Estreptomicinorresistentes

A ratones infectados con cinco distintas cepas estreptomicinorresistentes de bacilos tuberculosos, se les trató después con estreptomicina. Los resultados de estos experimentos demuestran que la estreptomicinoterapia no ejerció efecto nocivo. En tres de los cinco experimentos, los tiempos medios de sobrevivencia de los animales tratados con estreptomicina no discreparon mayor cosa de los tiempos medios en los animales testigos. En los otros dos experimentos, los tiempos medios de sobrevivencia de los ratones tratados con estreptomicina fueron significativamente mayores que en los testigos tratados con agua destilada. Esas diferencias en tiempos medios de sobrevivencia pudieron haber sido consecuencia de los efectos adversos del tratamiento prolongado con agua destilada.

Estudios cuantitativos del desarrollo *in vitro* revelan que el desarrollo de los bacilos tuberculosos resistentes a la estreptomicina no es realzado por la presencia de dicha droga. La estreptomicina, a concentraciones de 10 y 100 γ por ml. de medio, hizo bajar significativamente el índice aritmético de desarrollo, pero no ejerció efecto significativo sobre la fase logarítmica del desarrollo. Sin embargo, el índice logarítmico de desarrollo fué reducido significativamente por la estreptomicina a concentraciones de 1,000 γ por ml. de medio.

RESUME

L'action in vivo et in vitro de la streptomycine sur les bacilles tuberculeux streptomycino-résistants

Après inoculation avec cinq souches différentes de bacilles tuberculeux streptomycino-résistants, des souris ont été traitées par la streptomycine. Les résultats de cette expérimentation démontrent l'absence d'effets nocifs de la streptomycinothérapie. Dans trois des cinq études expérimentales, les temps moyens de survie des animaux traités par la streptomycine n'étaient pas augmentés de façon significative, par rapport à ceux des animaux témoins. Dans les deux autres études les temps moyens de survie étaient augmentés de façon significative, par rapport à ceux des animaux témoins traités avec de l'eau distillee. Il est possible que ces différences dans les temps moyens de survie résultent de l'effet nocif produit par un traitement prolongé avec de l'eau distillée.

Les études quantitatives *in vitro* sur la croissance prouvent que le développement du bacille tuberculeux streptomycino-résistant n'est pas renforcé par la présence de cet antibiotique. La streptomycine, aux concentrations de 10 et de 100 γ par ml. de milieu, a réduit dans des proportions significatives le taux arithmétique de la croissance, mais elle n'a pas modifié appréciablement la courbe logarithmique de croissance. Le taux logarithmique de croissance a été réduit par la streptomycine aux contrations de 1.000 γ par ml. de mlieiu.

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Case Reports

PULMONARY HISTOPLASMOSIS DIAGNOSED BY SCALENE NODE BIOPSY[1]

JOHN E. JOHNSON, JR., AND JOE M. MACCURDY

(Received for publication May 9, 1952)

INTRODUCTION

The relationship of histoplasmosis to pulmonary calcifications was postulated in 1945 (1), based on the observation that, in states bordering the Ohio River Valley and its tributaries, many persons with pulmonary calcification failed to react positively to the tuberculin skin test. Many of these were found to have a positive histoplasmin skin test. It was concluded that these states constituted an area endemic for a "benign" pulmonary form of histoplasmosis (2). Often no history of pulmonary disease could be elicited from these individuals, and there was no evidence of dissemination beyond the lungs.

Furcolow and his co-workers (3) were able to demonstrate a variety of miliary and nodular pulmonary lesions in Kansas City school children, associated with sensitivity to histoplasmin skin test and absent tuberculin hypersensitivity. After two to four years, these lesions proceeded to calcification with persistence of the skin test reactions (4). The calcified lesions ranged from large solitary to diffuse miliary in the form of their distribution through the lung fields. Furcolow (4, 5) reported the isolation of *Histoplasma capsulatum* from gastric washings of one such child with miliary lung lesions which later became calcified. No other cases of "benign" calcifications without other evidence of disease have come to our attention in which bacteriologic proof of the diagnosis of histoplasmosis was available.

Since organisms ordinarily cannot be demonstrated, the diagnosis of histoplasmosis in these cases must be made on an inferential basis. The essential criteria are the chest roentgenogram demonstrating pulmonary calcifications, positive reaction to the histoplasmin skin test, preferably a negative reaction to tuberculin, and residence in an endemic area.

Since Air Force recruits from the histoplasmosis endemic area are screened at Lackland Air Force Base, the medical staff there are often faced with the question of evaluating for service otherwise healthy young men with the clinical picture described above. The success and simplicity of the scalene node biopsy procedure in the diagnosis of sarcoidosis and lung carcinoma (6) suggested to the present writers that the procedure might be of value in these patients. The subject of the present report is a patient with apparently benign pulmonary histoplasmosis in whom the diagnosis was proved bacteriologically by study of material obtained through scalene node biopsy.

Case Report

C. G. N. was an 18-year-old white male who was asymptomatic prior to his referral to Lackland Air Force Base Hospital on November 20, 1951, because of findings on the routine medical processing chest roentgenogram. He specifically denied knowledge of any previous pulmonary disease other than minor upper respiratory infections. He had been living in a

[1] From the United States Air Force Hospital, Lackland Air Force Base, Texas.

small town in southwest Missouri and had previously lived on a farm in the same section of Missouri and in Ventura, California. His family had always kept a pet dog, and during the summer of 1951 he kept rabbits at his home. No definite history of recent or remote animal sickness to which he may have been exposed could be elicited. He had been doing construction work as a laborer prior to entering service.

Past history revealed that he had had diminished hearing of the left ear for many years. His last chest roentgenogram, which was made four years prior to service by a mobile unit surveying his school, was normal. Approximately three months prior to admission, the patient experienced a mid-abdominal pain following intermittent mild abdominal dis-

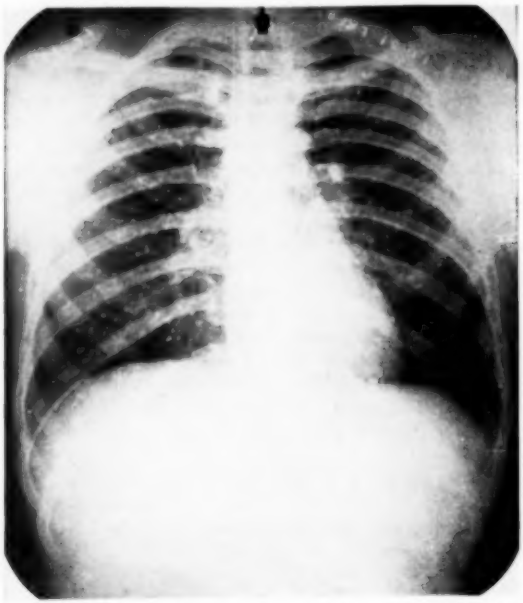

FIG. 1. Chest roentgenogram showing miliary calcifications and soft hilar lesions.

comfort for several days. The pain shifted to the right lower quadrant, with local tenderness and vomiting. This ceased after approximately six hours, prior to the arrival of his physician, who diagnosed appendicitis but did not recommend surgery. There have been no recurrences.

Physical examination revealed a healthy-appearing young male in no distress, with a temperature of 98.6°F. and a pulse rate of 78 and regular. Blood pressure was 108 systolic and 68 diastolic in mm. of mercury. Numerous postcervical nodes were palpable. A burn scar was present on the left anterior chest wall. Lung fields were entirely clear. The liver and spleen were not palpable. The ear, nose, and throat consultant reported that the patient had a conduction deafness on the left with retracted drum due to a blocked eustachian tube. Examination was otherwise negative.

Laboratory data: The original medical processing chest roentgenogram showed the lung fields to be studded with calcific densities measuring 1 to 3 mm. in diameter, with soft nodular prominence of both hilar shadows (figure 1). Repeat postero-anterior chest

films, made on November 23 and November 26, 1951, and on January 4, 1952, showed no change. On January 8, a right lateral film was made which provided no additional information. Hemogram and urinalysis were normal. The erythrocyte sedimentation rate (Wintrobe) was 11 mm. per hour. Cold agglutinin test was negative. Vital capacity was 102 per cent of normal. There was no sputum, but three gastric washings were negative on direct and concentrated smear and on culture for tubercle bacilli and for fungi. There was a one plus reaction to histoplasmin in dilution of 1:1,000 on two occasions, but no reaction to purified protein derivative (PPD) in first or second strength, or to coccidioidin.

The patient remained asymptomatic and afebrile until separated from the service, approximately three months after admission to the hospital. On December 7, 1951, a biopsy was performed under local anesthesia by Captain R. A. Breault to obtain the lymph nodes and fat pad lying on the right *Scalenus anticus* muscle, using the supraclavicular approach described by Daniels (6). The tissue for section proved to consist of several 1 to 3 mm. nodes embedded in fat. These were sectioned completely and interpreted as showing a moderately well-pronounced reticulum cell stroma with no abnormality.

A node measuring approximately 1 cm. in diameter was sliced and placed in modified Sabouraud's medium and in blood and blood cystine plates in parallel at 25° and 37°C. An organism with the morphologic characteristics of *Histoplasma capsulatum* was isolated. Growth occurred on only two plates, one blood cystine at 25° and one modified Sabouraud's at 37°C. The blood cystine plate was contaminated, and other plates showed no growth. The identity of the organism was confirmed by the Army Medical Service Graduate School, and the strain was shown to be virulent by mouse inoculation.

Subsequent cultures of bone marrow, urine, and stools were negative for *Histoplasma capsulatum*. Complement fixation performed by two different laboratories yielded conflicting results, but collodion agglutination test for *Histoplasma capsulatum* was negative.

COMMENT

The isolation of *Histoplasma capsulatum* from this patient appears to offer further support for the existence of benign pulmonary histoplasmosis. In the light of Raftery's (7) studies of intestinal histoplasmosis, it is possible that the patient's episode of "appendicitis" might represent evidence of more widespread infection. The presence of soft nodular prominences in the hilum is not the rule among persons with pulmonary calcifications. The incomplete calcification of the peripheral hilar lesions has been interpreted as indicating an infection of less than four years' duration, in the light of Bunnell and Furcolow's observations on the time required for calcification to be complete (5).

In the present case, no part of the single node from which a positive culture of *H. capsulatum* was obtained was subjected to histologic study. Two patients not reported here whose lesions gave the appearance of being incompletely calcified, one of whom had a palpable spleen, were subjected to right scalene node biopsy; the nodes were normal histologically but were not examined by bacteriologic techniques. These examples demonstrate the importance of dividing each separate specimen obtained by biopsy, giving half to the bacteriologist and half to the tissue pathologist (8).

Asymptomatic patients in whom pulmonary calcific lesions are found are given no treatment, and no restriction is placed on their activity. At this time the clinical significance of the positive node culture for this particular patient is problematical. Since disseminated histoplasmosis usually involves the lungs (9, 10), it is logical to assume that the lung may serve as the portal of entry for disseminated disease, in which event the persistence of viable virulent organisms within the body might be of serious prognostic import. The value of this procedure in pulmonary histoplasmosis is proved by this case; but the extent of its usefulness remains to be shown by the frequency with which a positive node biopsy and culture can be correlated with roentgenographic findings of calcification

and by follow-up observations to determine the frequency of subsequent dissemination of the disease. The implication of this consideration with reference to the screening of personnel recruited for military service remains to be defined.

SUMMARY

A case of multiple pulmonary calcifications in an asymptomatic young white male is presented in which a diagnosis of histoplasmosis was made on the basis of culture of a lymph node obtained from the surface of the *Scalenus anticus* muscle. The importance of culturing such biopsied material as well as studying it histologically is stressed.

SUMARIO

Histoplasmosis Pulmonar Diagnosticada por Biopsia de Ganglio Escaleno

En un caso de calcificaciones múltiples del pulmón en un joven blanco asintomático, hízose el diagnóstico de histoplasmosis a base de cultivos de un ganglio linfático obtenido de la cara del escaleno anterior. Recálcase la importancia que tienen el cultivo de ese material de biopsia así como el estudio histológico del mismo.

RESUME

Histoplasmose pulmonaire diagnostiquée par biopsie d'un ganglion lymphatique situé à la surface du scalène antérieur

Présentation d'un cas de calcifications pulmonaires multiples chez un jeune homme de race blanche. Le diagnostic d'histoplasmose est fondé sur la culture d'un ganglion lymphatique prélevé à la surface du scalène antérieur. L'importance de la culture d'un tissu de cette nature, obtenu par biopsie, ainsi que son étude histologique, est mise en évidence.

Acknowledgment

We wish to express our thanks to Captain John Stauch and Corporal Milton Altshuler for the bacteriologic studies, and to Lieutenant Colonel F. W. Wilson for his generous advice and criticism.

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HAZARDS OF ISONIAZID THERAPY IN EPILEPTICS[1]

Report of One Case

K. I. FETTERHOFF, C. X. HOLMES, AND G. E. MARTIN

(Received for publication June 20, 1952)

Isoniazid in toxic doses produces convulsions in all of the species of laboratory animals so far tested (1, 2); this convulsant action is diminished by the simultaneous administration of phenobarbital to the animals (3, 4). Severe toxic effects have not been reported in patients receiving the usual doses of isoniazid for the treatment of tuberculosis. The following case, however, shows that therapeutic doses of this compound can produce serious and even fatal episodes when administered to patients with epilepsy which is not completely controlled by the administration of barbiturates.

Case Report

A 43-year-old white male patient had suffered from jacksonian convulsive seizures for nineteen years following a head injury. During the year preceding the administration of isoniazid, the patient had averaged approximately one convulsive seizure each month, despite the intermittent administration of phenobarbital, 90 mg., and the regular administration of Dilantin, 0.2 gm. three times each day.

Four years prior to his death, the patient was found to have far advanced, bilateral, cavernous tuberculosis which progressed slowly despite treatment with bed rest, pneumothorax, streptomycin, and para-aminosalicylic acid (PAS). For this reason he was started on isoniazid, 100 mg., twice daily (3 mg. per kg. per day). The night following the institution of isoniazid therapy, he had one convulsion and, on the twelfth day of therapy, he had a second mild seizure. He last received phenobarbital, 90 mg., on the eighteenth day of therapy, and on the morning of the twenty-fourth day had an epileptic convulsion at 7:45 a.m. prior to receiving isoniazid that day. Other convulsions followed at 8:45, 9:00, and 9:30 a.m. Despite an attempt at therapy with sodium amytal and magnesium sulfate, the patient died, still convulsing, at 9:50 a.m.

During isoniazid therapy, this patient had felt somewhat better but had evidenced no hyperreflexia, muscular twitching, or euphoria. There were no other known factors which might have contributed to the production of status epilepticus.

SUMMARY

The fatal outcome in this case serves to emphasize the necessity for careful control of epilepsy with phenobarbital prior to and during the use of isoniazid in epileptic patients.

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CHEST SURVEY ON PRIVATE PATIENTS: SOME PROBLEMS

To the Editors of the American Review of Tuberculosis:

Experience with chest survey or miniature film examination of the chest on private patients has been limited in the experience of most radiologists. As a procedure to complete the medical examination, radiologic study of the chest is of value. However, the drawbacks of the use of chest survey on private patients and the abuses of it, as well as the economic factors that fail to operate if the numbers of patients handled are not large, make this procedure impractical for the average radiologist. It may also contribute to the belief that the chest examination which may encompass fluoroscopy, kymography, a swallow of barium, and five to seven (or even more) films at various angles can be done on the same economic outlay.

The disservice that chest-survey type of examinations may do to the correct medical (radiologic) diagnosis of chest disease is not known, but may place the routine chest examination in a poor light. An attempt to compare economically chest-survey and individual chest examination has already led to considerable restiveness on the part of some private radiologists and made them try to compete with the chest-survey type of examination by lowering their prices for routine chest examinations. This is fraught with economic difficulties for the normal volume practice of radiology. This is an artificial attempt to meet a pressure which all of modern medicine is feeling. The radiologist of today must do a much larger volume of work (and does) than his confrere of twenty-five or thirty years ago to make a comparable dollar income, but the numbers of dollars of income are approximately the same although the value of the dollar has obviously not remained the same. The wedge of the chest survey lowering the charge for chest examination is erroneous and subject to much more scrutiny than has been given in the past.

The radiologist is faced with being called to task for delaying the use of the chest survey (case-finding technique) on private patients, but, as is true in practically every specialty, his confrere of another specialty fails to appreciate the difficulties of that specialty, not only in its type of medical practice, but also in its economics.

As one who has had four and one-half years' experience with chest survey on private patients, I believe that at this time we have approached economic stability in this examination for the first time. Prior to this time we have been in a position of being economically unsound. This is with a present volume of approximately fifty patients a day. With approximately half this number, the calculated costs would not allow us to carry the equipment and processing and handling of the films. The radiologist's time (especially when double reading is done, as we do) has not been counted in, and the amount of time spent in governing this unit has been considerable. The situation in which some of us practice in groups is unique and usually does not apply to the radiologist in private practice. The use of minifilms as diagnostic (not survey) examinations has steadily increased; this has increased the handling costs an intangible but obviously considerable amount.

It is my opinion that, if radiologists in private practice embark upon a "cut-rate" chest examination as a part of their routine, they will find that it is economically and medically unsound.

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SIDNEY F. THOMAS

May 26, 1952

American Trudeau Society

Medical Section of the National Tuberculosis Association

Report of the Committee on Medical Research—1951–1952

The Committee on Medical Research met twice during the year, on October 10 to 12, 1951, in New York and on May 23, 1952, in Boston, Massachusetts. The principal business of the meetings was to review the work of investigators receiving medical research grants from the National Tuberculosis Association and to select the most worthy candidates from among the applicants seeking such support for future investigation. The latter task was especially difficult this year because the number of applications was much greater than in previous years, while the sum available for grants was not. The Committee reviewed each application with care, making the best possible choice on the merits of the proposed studies and the demonstrated ability of the investigators who would carry them out.

Thirty-four grants at a total cost of $199,056 were recommended for the approval of the National Tuberculosis Association Board of Directors for the fiscal year 1952 to 1953. The titles of the research projects and the names of the investigators are listed following this summary.

One of the high points of the year's activities was the annual joint meeting of the Committee with the investigators currently receiving grants. At this meeting each investigator reports in person on the progress of his work. Thus, the Committee learns the status of each study and is able to survey the research program in its entirety. Bringing together many experts in various fields, the session also affords an interchange of ideas on current problems of research that is of inestimable value to all present.

The Committee fulfilled a request of the Laboratory Subcommittee, which was approved by the American Trudeau Society Council, to collect information on the availability of BCG vaccine in the United States and procedures for procuring the vaccine for clinical and experimental use. Statements from the directors of the three laboratories manufacturing and distributing the vaccine were published in the May 1952 issue of THE AMERICAN REVIEW OF TUBERCULOSIS.

The Committee also considered the recommendation of the Laboratory Subcommittee for the possible need for revision of the National Institutes of Health booklet *Minimum Requirements: BCG Vaccine* (1949) in the light of a number of developments in the preparation and use of the vaccine. It was thought that participation of the Committee might be invited for a revision, as in the original preparation of the standards now in use. The Committee suggested that the President of the American Trudeau Society appoint a small subcommittee of experts in the field of immunology to supply such advice as may be requested.

Another function of the Committee on Medical Research during the year was to review the work of its subcommittees, the Laboratory Committee and the Fellowship Board, and the closely related Committee on Therapy. The work of the three committees had the hearty support of the parent committee, but, as the work of these committees is to be reported separately, a description of the work of those committees is not neces sary here.

Research Grants Approved for 1952 to 1953

Investigator	*Institution*	*Subject*
Rudolph J. Anderson	Yale University	Chemistry of tubercle bacilli
Theodore L. Badger	Boston City Hospital	Studies in pulmonary physiology

Investigator	*Institution*	*Subject*
*Walter L. Bloom and Martin M. Cummings	Emory University; Lawson Veterans Administration Hospital	Mechanisms of natural resistance to tuberculosis
Vernon Bryson	Biological Laboratory, Cold Spring Harbor, New York	Genetics of *Mycobacteria*
Paul A. Bunn	State University of New York	Effect of adrenal hormones on ocular tuberculosis in the rabbit treated with antituberculous drugs
Mary I. Bunting	Yale University	Genetics of *Mycobacteria*
* John W. Berry	University of Colorado, Denver, Colorado; and Colorado Foundation for Research in Tuberculosis, Colorado Springs, Colorado	Antibiotics and other chemicals in tuberculosis
Amos Christie	Vanderbilt University	Pulmonary calcification in fungus diseases
Sidney P. Colowick and Nathan O. Kaplan	McCollum-Pratt Institute; Johns Hopkins University	Mechanism of action of isonicotinic acid derivatives on tubercle bacilli
Bruno Gerstl	Veterans Administration Hospital, Oakland, California	Factors influencing the occurrence of humoral antibodies in tuberculosis
K. Albert Harden	Howard University	Chronic pulmonary disease, lung surgery and cardiac function
Waldemar F. Kirchheimer	Northwestern University	Transfer of tuberculin-type sensitivity with cell-free material and nature of the tuberculin antibody
Melvin H. Knisely	Medical College of State of South Carolina	Correlation of intravascular sludge formation, sedimentation rate, and clinical progress in tuberculosis
Vernon E. Krahl	University of Maryland	Structure of mammalian lung
Edith M. Lincoln	New York University-Bellevue Medical Center	Tuberculosis in children
Max B. Lurie	Henry Phipps Institute, University of Pennsylvania	Genetic resistance to tuberculosis
Walsh McDermott	Cornell University	Host-parasite relationships and drug treatment in tuberculosis
Roger S. Mitchell and Gordon M. Meade	Trudeau Sanatorium	Statistical study of various forms of treatment in tuberculosis

Investigator	*Institution*	*Subject*
Carroll E. Palmer	Division of Chronic Disease and Tuberculosis, Public Health Service, Washington, D. C.	Minimal tuberculosis in nurses
Emanuel M. Papper	Columbia University	Effects of anesthesia during pulmonary resection
Hilda P. Pope	Duke University	Metabolism of tubercle bacilli
Sidney Raffel	Stanford University	Immunity and allergy in tuberculosis
Paul B. Sawin	Jackson Memorial Laboratory, Bar Harbor, Maine	Propagation of rabbit stocks of known genetic resistance to tuberculosis
Florence B. Seibert	Henry Phipps Institute, University of Pennsylvania	Antigens of tubercle bacillus; relationship of their chemical structure to biological potency
William Steenken, Jr.	Trudeau Laboratory	Maintenance of Culture Depot for tubercle bacilli
Arthur J. Vorwald	Saranac Laboratory	Effect of silica on tuberculosis caused by attenuated tubercle bacilli
Dwain N. Walcher	Indiana University Medical Center	Evaluation of streptokinase-streptodornase in tuberculous meningitis
Charles Weiss	Albert Einstein Medical Center, Northern Division	Caseation and softening in tuberculosis
James L. Whittenberger	Harvard School of Public Health	Basic studies on the mechanics of breathing in man
H. Stuart Willis	North Carolina Sanatorium	Studies in vaccination against tuberculosis
Richard J. Winzler	University of Southern California	Mucoproteins in human plasma
C. Eugene Woodruff	Wm. H. Maybury Sanatorium	Intraperitoneal vaccination of guinea pigs by heat-killed tubercle bacilli
George W. Wright	Trudeau Sanatorium	Pulmonary function studies
National Society for Medical Research		Contribution

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DAVID T. SMITH, *Chairman*

Report of the Fellowship Board of the Committee on Medical Research—1951–1952

Two meetings of the Fellowship Board were held during the year, one on October 10, 1951, in New York and one on May 23, 1952, in Boston, Massachusetts. At these meetings 17 research fellowships at a cost of $47,300 were recommended and, by previous arrangement with the Committee on Medical Education, two teaching-resident fellowships at a cost of $5,800 were also recommended. The names of the Fellows, their sponsors, and the institutions where they will work are listed below:

Fellow	*Supervisor*	*Institution*
Elizabeth P. Anderson	Charles Weiss	Albert Einstein Medical Center, Northern Division
Johannes Bartels	Julius B. Comroe, Jr.	University of Pennsylvania
Donald D. Daniels	T. L. Althausen and Ellen Brown	University of California
Arthur M. Dannenberg, Jr.	Max B. Lurie	Henry Phipps Institute, University of Pennsylvania
Seymour Froman	Emil Bogen	University of California at Los Angeles
L. Ruth Guy	Sidney Raffel	Stanford University
Rosemarie Haarburger	Edith M. Lincoln	New York University-Bellevue Medical Center
Jean Ellen Hawkins	David T. Smith	Duke University
Nelda B. Holmgren	Guy P. Youmans	Northwestern University
Edward M. La Fond	W. B. Tucker, Edward T. Evans, Russell H. Frost, Ejvind P. Fenger	Glen Lake Sanatorium
Leroy C. McLaren	G. J. Jann	University of California at Los Angeles
Quentin N. Myrvik	Russell S. Weiser	University of Washington
Ralph G. Spears	Guy P. Youmans	Northwestern University
Helen Wago	Vera S. Fry	New York University
Joseph W. Whalen	W. L. Mallman	Michigan State College
National Research Council (2)	Milton C. Winternitz	As appointed
	Teaching-Resident Fellowships	
J. Park Biehl	John H. Skavlem and M. H. Blankenhorn	University of Cincinnati
Stanley Spellman	Robert G. Bloch	Montefiore Hospital

Other business taken up by the Fellowship Board during the year included revision of the fellowship application forms and discussion of a resolution on teaching appointments in pulmonary diseases adopted by the Committee on Medical Education in October, 1951. This resolution recommended that the Fellowship Board consider the allocation of funds to subsidize (wholly or in part) teaching appointments in pulmonary diseases

more advanced than research fellowships in both academic position and salary. This was approved in principle by the Fellowship Board and by the Committee on Medical Research. Exploration of local sources was encouraged, however, as it would be impossible for the National Tuberculosis Association to assume responsibility for the enlarged program with funds presently at its disposal. It was suggested that the question be given further consideration when the proposed Division of Professional Education has been organized within the American Trudeau Society.

Reuben J. Erickson
Russell H. Frost
Julius L. Wilson
H. McLeod Riggins

H. Corwin Hinshaw, *Chairman*

NOTES

ANNUAL MEETING OF AMERICAN TRUDEAU SOCIETY—1953

The American Trudeau Society, medical section of the National Tuberculosis Association, invites the submission of abstracts of scientific papers for presentation at its next annual meeting in Los Angeles, California, May 18 to 22, 1953, at the Hotel Statler.

Full information can be obtained by writing to the Chairman of the Medical Sessions Committee, American Trudeau Society, 1790 Broadway, New York 19, N. Y.

TUBERCULOSIS NURSING ADVISORY SERVICE

Since the National League of Nursing Education, the National Organization for Public Health Nursing, and the Association of Collegiate Schools of Nursing are now one organization—the National League for Nursing—the "Joint" in the title of the Joint Tuberculosis Nursing Advisory Service no longer applies. The name of this service has therefore been changed to the Tuberculosis Advisory Nursing Service (TANS)[1] of the National League for Nursing (NLN).

Miss Shelia M. Dwyer is the consultant in nursing education and hospital service and Miss Jean South is the public health nursing consultant in education and service.

[1] TANS is made possible through a grant from the National Tuberculosis Association.

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*Wilbur, D. L.: Principles in the Use of Vitamins in Treatment: I. Vitamin Deficiency Diseases. Gastroenterology, 1:179, Feb., 1943.

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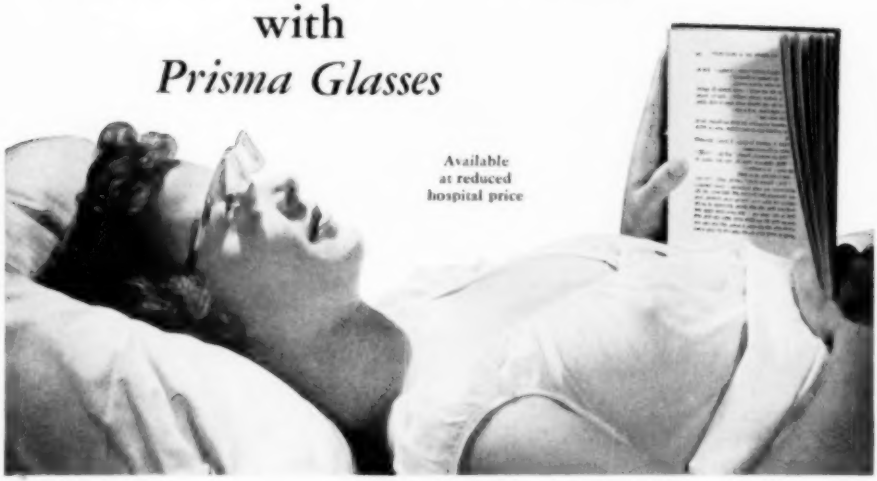

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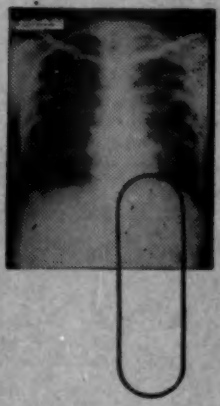

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Each capsule-shaped, uncoated tablet contains 0.5 Gm. Para-Aminosalicylic Acid in a special acid-buffering system.

THE Panray CORP.

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